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Antioxidant properties of fermented soybean broth

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Abstract

Soybean broth (SB) and fermented soybean broth (FSB), at 100% exhibited good antioxidant activities of 0.85 and 0.80, respectively. Only FSB exhibited an excellent reducing power of 0.76–0.86 at 5–100%. FSB showed an excellent scavenging activity on 1,1-diphenyl-2-picrylhydrazyl radicals (100%) at 20–100%, while SB only at 100%. FSB at 20–100% inhibited the production of superoxide anion radicals by 81–93% but SB showed no inhibition. As its concentrations increased from 2–100%, the scavenging effect of FSB on hydroxyl radicals increased from 30–96%, whereas that of SB increased from 5–76%. Both FSB and SB were good chelators for ferrous ions. For cupric ions, SB showed significantly higher chelating effect than did FSB. These results showed that FSB was superior to SB in most antioxidant properties and that SB and FSB, more specifically FSB, might be potential antioxidants for application in food products.

Keywords: Fermented soybean broth; Antioxidant activity; Reducing power; Scavenging effect; Chelating effect

1. Introduction

Highly reactive free radicals, especially oxygen-derived radicals, which are formed by exogenous chemicals or endogenous metabolic processes in the human body or in food systems, are capable of oxidizing biomolecules, resulting in cell death and tissue damage. Oxidative damage plays a significantly pathological role in human diseases. Cancer, emphysema, cirrhosis, atherosclerlosis, and arthritis have all been correlated with oxidative damage (Halliwell & Gutteridge, 1984). Almost all organisms are well protected against free radical damage by enzymes such as superoxide dismutase and catalase, or compounds such as ascorbic acid, tocopherol and glutathione (Niki, Shimaski & Mino, 1994). When the mechanism of antioxidant protection becomes unbalanced by factors such as aging, deterioration of physiological functions may occur, resulting in diseases and accelerating aging. However, antioxidant supplements or foods containing antioxidants may be used to help the human body reduce oxidative damage.

Synthetic antioxidants are widely used because they are effective and cheaper than natural types. However, the safety and toxicity of synthetic antioxidants have been important concerns (Imaida, Fukushima, Shivai, Ohtani, Nakanishi & Ito, 1983). Much attention has been focused on the use of antioxidants, especially natural antioxidants, to inhibit lipid peroxidation or to protect the human body from the oxidative damage by free radicals.

Soybean is the most important legume and the major source of protein in the traditional Chinese diet. In addition, soybean contains many nutritional components. Generally, a fermented soybean broth is thought to be a healthy drink in Taiwan. However, the beneficial effects of this fermented soybean product are unknown, especially its antioxidant properties and scavenging effects on reactive oxygen. Our objective was to evaluate the antioxidant properties of this fermented product, including antioxidant activity, reducing power, scavenging effects on radicals, and chelating effects on metallic ions. A comparison with those of an unfermented product was also made.

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Abbreviations: AOA, antioxidant activity; BHA, butylated hydroxyanisole; DMPO, 5,5-dimethyl pyrroline-N-oxide; DPPH, 1,1-diphenyl-2-picrylhydrazyl, EPR, electron paramagnetic resonance, FSB, fermented soybean broth, NADH, nicotinamide adenine dinucleotide; NBT, nitro blue tetrazolium; PMS, phenazine methosulfate; SB, soybean broth, TMM, tetramethyl murexide

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2. Materials and methods

2.1. Fermented soybean broth

Defatted soybean meal (10%) was suspended in an aqueous solution supplemented with 1% cane molasses. The suspension was inoculated with a culture mixture consisting of *Acetobacter* sp., *Lactobacillus* sp., *Saccharomyces* sp. and *Streptomyces* sp., and then incubated at 20°C for 21 days. The fermented suspension was autoclaved and filtered through cheesecloth. The broth thus obtained was referred to as fermented soybean broth (FSB), and diluted into various concentrations (100, 50, 20, 10, 5 and 2%) to analyze the profile of its antioxidant properties. In addition, soybean broth (SB) was prepared by following the previous procedure except for the culture inoculation and 21-day fermentation. One natural antioxidant, α-tocopherol (Sigma Chemical Co., St. Louis, MI), and one synthetic antioxidant, butylated hydroxyanisole (BHA, Sigma), were also used at 20 mM as controls.

2.2. Antioxidant activity

After dilution with deionized water, various concentrations of FSB or SB (0.1 ml) were mixed with linoleic acid (Sigma) emulsion (2 ml, 10 mM, pH 6.5) in test tubes and placed in darkness at 37°C to accelerate oxidation. After incubation for 15 h methanol (Mallinckrodt Baker, Paris, KY) in deionized water (6 ml, 60%) was added, and the absorbance of the mixture was measured at 234 nm in a Hitachi U-2001 spectrophotometer. The antioxidant activity (AOA) was calculated according to Lingnert, Vallentin and Eriksson (1979). AOA = (ΔA_{234} of control − ΔA_{234} of sample)/ΔA_{234} of control. An AOA value of 1 indicates the strongest antioxidant activity. The linoleic acid emulsion was prepared by dissolving 0.2804 g linoleic acid and 0.2804 g Tween 20 (Sigma) in 0.2 M sodium phosphate buffer (50 ml, pH 6.5) and homogenizing in a Kontes Dual tissue grinder to form an emulsion.

2.3. Reducing power

The reducing power of FSB or SB was determined according to the method of Oyaizu (1986). Various concentrations of FSB or SB (2.5 ml) were mixed with sodium phosphate buffer (2.5 ml, 200 mM, pH 6.6, Wako Pure Chemical, Osaka, Japan) and 1% potassium ferricyanide (2.5 ml, Sigma), and the mixture was incubated at 50°C for 20 min. After trichloroacetic acid (2.5 ml, 10%, w/v, Wako) was added, the mixture was centrifuged at 650 *g* for 10 min. The upper layer (5 ml) was mixed with deionized water (5 ml) and 0.1% ferric chloride (1 ml, Wako), and the absorbance was measured at 700 nm. A higher absorbance indicates a higher reducing power.

2.4. Scavenging effect on 1,1-diphenyl-2-picrylhydrazyl radicals

Various concentrations of FSB or SB (4 ml) were mixed with 1 ml of methanolic solution containing 1,1-diphenyl-2-picrylhydrazyl (DPPH, Sigma) radicals, such that the final concentration of DPPH was 0.2 mM. The mixture was shaken vigorously and left to stand for 30 min, and the absorbance was then measured at 517 nm (Shimada, Fujikawa, Yahara & Nakamura, 1992).

2.5. Scavenging effect on superoxide anion radicals

Superoxide anion was determined using spectrophotometric measurement of various concentrations of FSB or SB on the reduction of nitro blue tetrazolium (NBT) (Robak & Gryglewski, 1988). Superoxide anion was generated in a non-enzymatic phenazine methosulfate–nicotinamide adenine dinucleotide (PMS–NADH) system. The non-enzymatic generation of superoxide anion was measured in reaction mixtures containing various concentrations of FSB or SB, PMS (60 μM, Sigma), NADH (468 μM, Sigma) and NBT (150 μM, Sigma) in phosphate buffer (0.1 M, pH 7.4). After incubation for 5 min at ambient temperature, the color was read at 560 nm against blank samples.

2.6. Scavenging effect on hydroxyl free radicals

The hydroxyl radical reacted with the nitrone spin trap 5,5-dimethyl pyrroline-*N*-oxide (DMPO, Sigma) and the resultant DMPO-OH adduct was detected with an electron paramagnetic resonance (EPR) spectrometer. The EPR spectrum was recorded 2.5 min after mixing various concentrations of FSB or SB (0.2 ml) with H_2O_2 (0.2 ml, 10 mM, Merck, Darmstadt, Germany), Fe^{2+} (0.2 ml, 10 mM, Sigma) and DMPO (0.2 ml, 10 mM) using an EPR spectrometer (Bruker EMX-10) set at the following conditions: 3480-G magnetic field, 1.0 G modulation amplitude, 0.5 s time constant, and 200 s scan period (Shi, Dalal & Jain, 1991).

2.7. Chelating effects on ferrous and cupric ions

Chelating effect was determined according to the method of Shimada et al. (1992). To 2 ml of the mixture, consisting of 30 mM hexamine (Wako), 30 mM potassium chloride (Sigma) and 9 mM ferrous sulfate (or copper sulfate, Union Chemical Works, Hsinchu, Taiwan) various concentrations of FSB or SB (2 ml) and tetramethyl murexide (TMM, 0.2 ml, 1 mM, Sigma) were added. After 3 min at room temperature, the

absorbance of the mixture was determined at 485 nm. A lower absorbance indicates a higher chelating power.

2.8. Statistical analysis

For FSB or SB, three samples were prepared for assays of every antioxidant attribute. The experimental data were subjected to an analysis of variance for a completely random design as described by Steel, Torrie and Dickey (1997) to determine the least significant difference at the level of 0.05. In the following figures, the plotted means with the same capital (for ferric ions) or small letter within a given concentration are not significantly different at the level of 0.05.

3. Results and discussion

3.1. Antioxidant activity and reducing power

FSB and SB at 100% exhibited good antioxidant activities of 0.80 and 0.85, respectively, i.e. 80 and 85% inhibition of peroxidation of linoleic acid (Fig. 1), i.e. lower than that of 20 mM BHA (0.94), and comparable to that of 20 mM α-tocopherol (0.83). The antioxidant activity of various concentrations of FSB and SB reduced gradually with the increased dilution to 10% and then steeply dropped to 2%. However, at concentrations from 10 to 100%, FSB and SB were comparable in antioxidant activity ranging from 0.51 to 0.85. This indicated that both FSB and SB inhibited peroxidation of linoleic acid at concentrations as low as 10%, regardless of fermentation. The antioxidant activity of FSB and SB might be due to the reducing of hydroperoxide, inactivating of free radicals, or complexing with metal ions, or combinations thereof. This good antioxidant activity of FSB and SB might be attributed to the presence of phytochemicals, such as isoflavones (Wang and Wixon, 1999).

The reducing power of FSB (absorbance at 700 nm) remained at 0.76 to 0.86 at concentrations from 5 to 100%, and dropped to 0.61 at 2% (Fig. 2). The reducing power of SB was 0.43 at 100%, and dropped to the level of 0.12 to 0.04 at concentrations from 50 to 2%. However, at 20 mM, the reducing powers of BHA and α-tocopherol were only 0.12 and 0.13, respectively. Evidently, only FSB exhibited excellent reducing power. FSB might produce certain metabolites with superior reducing power during fermentation, creating a major discrepancy between FSB and SB.

The antioxidant activity has been reported to be concomitant with the development of reducing power (Tanaka, Kuie, Nagashima & Taguchi, 1988). However, this pattern was not observed in this research. Okuda , Kimura, Yoshida, Hatano, Okuda and Arichi (1983) reported that the reducing power of tannins prevents liver injury by inhibiting the formation of lipid peroxides. Furthermore, reductones, such as ascorbic acid, can react directly with peroxides and also with certain precursors and thereby, prevent peroxide formation (Shimada et al., 1992). The reducing power of FSB might be due to its hydrogen-donating ability, as described by Shimada et al. (1992). Therefore, FSB might contain reductones formed during fermentation, which could react with free radicals to stabilize and terminate radical chain reactions.

3.2. Scavenging effects on free radicals

FSB showed an excellent scavenging activity on DPPH radicals (100%) at concentrations from 20 to 100% (Fig. 3). SB showed 100% scavenging activity only at 100%. The scavenging activities of SB decreased gradually with increased dilution to 20% and then rapidly dropped to 2%. At 20 mM, the scavenging

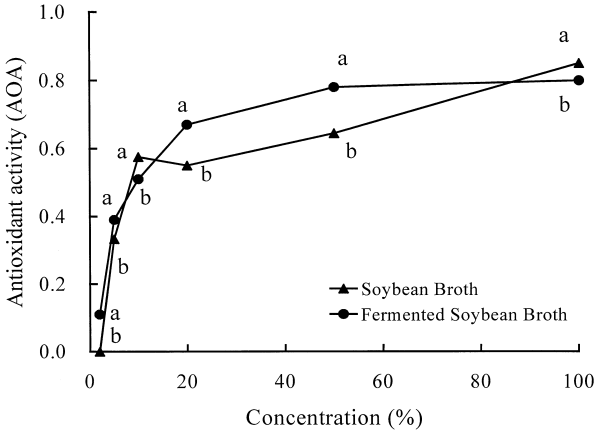

Fig. 1. Antioxidant activity of soybean broth and fermented soybean broth. Values with the same letter within a given concentration are not significantly different at the level of 0.05.

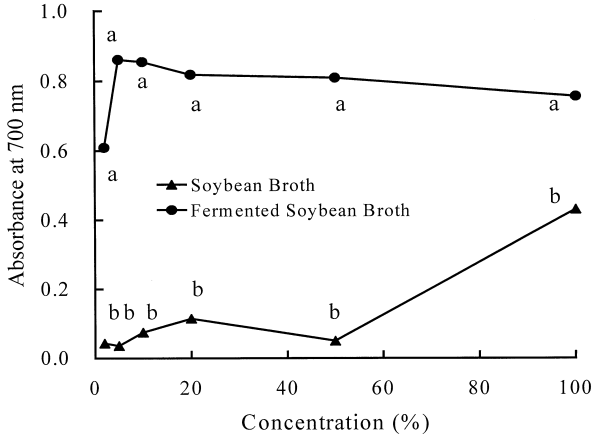

Fig. 2. Reducing power of soybean broth and fermented soybean broth. Values with the same letter within a given concentration are not significantly different at the level of 0.05.

activities of BHA and α-tocopherol were 0.96 and 0.95, respectively. Obviously, there were more antioxidant components present in FSB than in SB, which could react rapidly with DPPH radicals, and reduce almost all DPPH radical molecules corresponding to available hydroxyl groups (Brand-Williams, Cuvelier & Berset, 1995). This result reveals that FSB and SB are free radical inhibitors or scavengers, acting possibly as primary antioxidants. FSB and SB might react with free radicals, particularly of the peroxy radicals, which are the major propagatous of the autoxidation chain of fat, thereby terminating the chain reaction (Frankel, 1991; Gordon, 1990; Shahidi & Wanasundara, 1992). Antioxidant activity of natural antioxidants has been shown to be involved in termination of free radical reactions and reducing power (Shimada et al., 1992; Tanaka et al., 1988). Based on the results obtained, the marked inhibitory effect of FSB in peroxidation of linoleic acid could in part be caused by its properties of scavenging free radicals and containing reductones. However, the inhibitory effect of SB could in part be caused by its scavenging properties.

FSB at concentrations from 20 to 100% inhibited the production of superoxide anion radicals by 81 to 93% (Fig. 4). FSB at 2% showed a moderately inhibitory effect on superoxide anion production (59%). However, BHA and α-tocopherol at 20 mM and SB, at all concentrations tested, showed completely no inhibition. Superoxide anions indirectly initiated lipid oxidation as a result of superoxide and hydrogen peroxide serving as precursors of singlet oxygen and hydroxyl radicals (Aurand, Boonme & Gidding, 1977). Robak and Gryglewski (1988) reported that the antioxidant properties of flavonoids are effective mainly via the scavenging of superoxide anion. Furthermore, soybean was reported to contain certain amount of isoflavones, such as daidzein and genistein (Rajalakshmi & Narasimhan, 1996), which are also antioxidants and might possess the scavenging effect similar to flavonoids. However, Esaki, Onozaki and Osawa (1994) found that the contents of these isoflavones only slightly increased in fermented soybean products.

As its concentrations increased from 2 to 100%, the scavenging effect of FSB on hydroxyl radicals in electron paramagnetic resonance spectra increased from 30 to 96%, whereas that of SB increased from 5 to 76% (Fig. 5). Interestingly, these two curves were parallel. However, at 20 mM, the scavenging effects of BHA and α-tocopherol were 23 and 34%, respectively. Okamoto, Hayase and Kayo (1992) indicated that glycated protein had a scavenging ability for hydroxyl radicals. Shi et al. (1991) reported scavenging activity of hydroxyl radicals of caffeine, and attributed the alleged anticarcinogenic properties of caffeine to this activity. Furthermore, Yen and Hsieh (1995) pointed out that the ability of xylose-lysine Maillard reaction products to quench the hydroxyl radicals was directly related to their antimutagenicity. Therefore, it was anticipated that FSB might possess much more effective antimutagenic properties than SB.

3.3. Chelating effects on metal ions

The chelating effects on ferrous ions were excellent for FSB from 100 to 50% and for SB from 100 to 20% (Fig. 6). After extensive dilution to 5 and 2%, the chelating effect on ferrous ions were reduced to the range 53–61%. This indicated that both FSB and SB were good chelators for ferrous ions. For cupric ions, SB showed significantly higher chelating effect than did FSB. Apparently, the chelating effect of FSB was better on ferrous ions than on cupric ions, whereas FSB possessed superior chelating capability on ferrous ions over cupric ions. At 20 mM, the chelating effects of BHA on ferrous and cupric ions were 0.36 and 0.24,

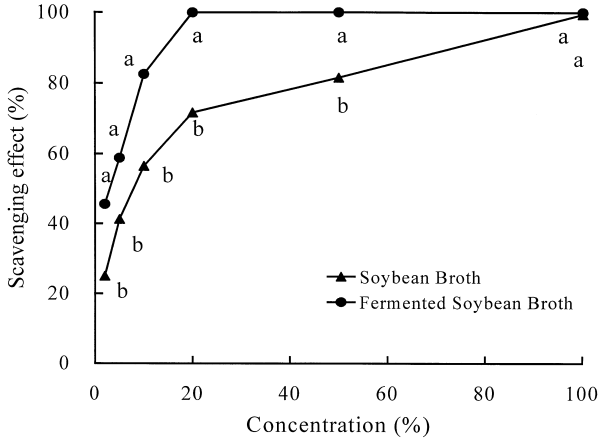

Fig. 3. Scavenging effect of soybean broth and fermented soybean broth on 1,1-diphenyl-2-picrylhydrazyl radical. Values with the same letter within a given concentration are not significantly different at the level of 0.05.

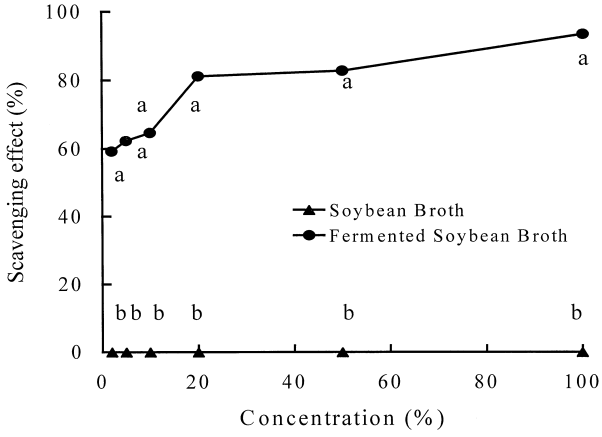

Fig. 4. Scavenging effect of soybean broth and fermented soybean broth on superoxide anion. Values with the same letter within a given concentration are not significantly different at the level of 0.05.

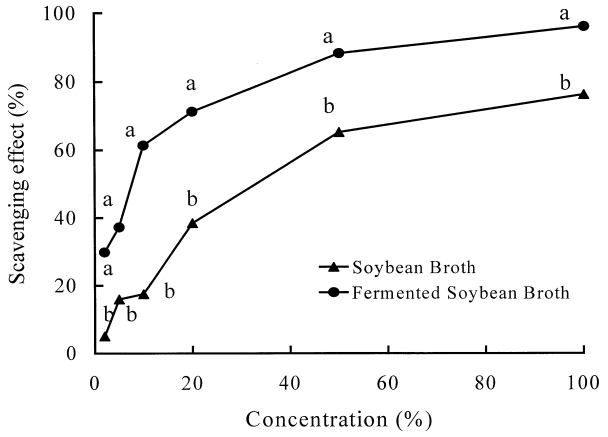

Fig. 5. Scavenging effect of soybean broth and fermented soybean broth on hydroxyl free radical. Values with the same letter within a given concentration are not significantly different at the level of 0.05.

whereas those of α-tocopherol were 0.92 and 0.99, respectively. Since ferrous and cupric ions are the most effective pro-oxidants in food systems (Yamaguchi, Tatsumi, Kato & Yoshimitsu, 1988), and ferrous ions are commonly found in foods systems, the higher chelating effects of SB and FSB would be beneficial.

These results clearly showed that SB possessed good antioxidant properties, including antioxidant activity, scavenging abilities on DPPH and hydroxyl radicals and chelating abilities on both ferrous and cupric ions. As compared to SB, FSB exhibited comparable antioxidant activity and ferrous chelating capability, and better scavenging effects on DPPH, superoxide anion and hydroxyl free radicals, but less cupric chelating capability. SB and FSB both acted as both primary antioxidant and oxygen scavengers. It is obvious that the fermentation of soybean broth could result in better antioxidant properties, except for the cupric chelating effect. However, BHA and α-tocopherol showed excellent antioxidant properties only in antioxidant activity and scavenging effect on DPPH radicals. In addition, α-tocopherol exhibited better chelating effects on both ions.

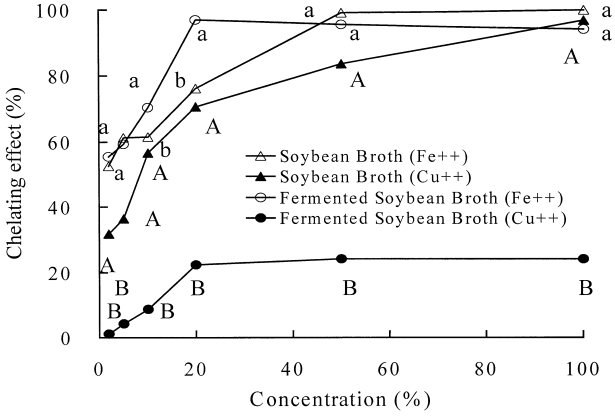

Fig. 6. Chelating effect of soybean broth and fermented soybean broth on ferrous and cupric ions. Values with the same capital (Fe^{2+}) or small letter (Cu^{2+}) within a given concentration are not significantly different at the level of 0.05.

Like soymilk, SB is considered to be a nutritive drink in Taiwan. In this research, the findings of antioxidant properties of SB could inevitably expand its application and consumption. In addition, FSB was superior to SB in most antioxidant properties. SB and FSB (more specifically FSB) might be potential antioxidants for application in food products. For application in the food industry or for the study of the antioxidant mechanism, further research on the isolation of the antioxidant components in both SB and FSB is in progress.

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