Food Sci. Technol. Res., **10** (3), 324–327, 2004

Note

Radical-Scavenging Activity and Isoflavone Content of Sufu (Fermented Tofu) Extracts from Various Regions in China

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Received February 23, 2004; Accepted May 12, 2004

Water extracts and 50% ethanol extracts of 14 samples of sufu (fermented tofu) produced in various regions of China were prepared, and their DPPH (α,α-Diphenyl-β-pricryl-hydrazyl) radical-scavenging activity and isoflavone content were determined. Although the extracts showed different radical-scavenging activity levels depending on their color and region of production, all contained highly active components. Radical-scavenging activity in the water extracts ranged from 2.03 to 11.93 μg α-tocopherol/mg, while that for the 50% ethanol extracts ranged from 2.14 to 14.62 μg α-tocopherol/mg. Radical-scavenging activity in extracts from grey sufu was higher than that found in red or white sufu. Samples from the same regions showed similar radical-scavenging activity, but there was no clear directional tendency (north/south, east/west) for the produced sufu to have high activity. Most of the isoflavone in sufu was shown to be in the form of aglycone. Isoflavone aglycones in water extracts ranged from 50.2 to 179.3 μg/g of dry matter, and that in 50% ethanol extracts ranged from 199.0 to 706.9 μg/g of dry matter.

Keywords: radical-scavenging activity, sufu, isoflavone

Introduction

Sufu is a traditional fermented soybean curd resembling a soft creamy cheese-type product that has been widely consumed by Chinese people for more than 1,000 years (Han *et al.*, 2001a). Sufu is produced by a variety of processes depending on the region of production. While there has been research on the general processing of sufu including its microbiological and biochemical characteristics (Han *et al.*, 2001b and 2003), the quality and function of local types of sufu in various regions are still not clear.

Recently, more and more people are interested in physiological properties of foods such as antioxidative and anticancer activity. Many types of fermented soybean foods have been reported to exhibit much stronger antioxidative activity than unfermented soybeans (Fukushima, 2001). Fermented soybean broth has shown greater radical scavenging avtivity on α,α-Diphenyl-β-pricrylhydrazyl (DPPH) than unfermented soybean broth (Yang *et al.*, 2000). Wang *et al.* (2003) were the first to report on the antioxidative activity of sufu. It was also reported that soybean isoflavones had a preventive effect on the development of some kinds of cancer (Adlercreutz *et al.*, 1992). Isoflavone contents and composition of sufu were shown to change during the fermentation (Li *et al.*, 2003). However, the relationship between the manufacturing procedure and the physiological properties of sufu has not been systematically studied yet.

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In this stduy, we analyzed the radical-scavenging activity of water or 50% ethanol extracts of sufu from various regions of China. We also determined the content and composition of isoflavones in the extracts. By clarifying the relationship between the function and characteristics of sufu, it might be possible to establish improved processing for highly active functional sufu and increase its value as a food product.

Materials and Methods

Materials Fourteen samples of sufu were obtained from local markets in 10 different regions of China. 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2-morpholinoethanesulfonic acid monohydrate (MES) and α-tocopherol were purchased from Wako Pure Chemical Co., Ltd. (Osaka, Japan). Standard daidzin, genistin, glycitin, daidzein, genistein, glycitein were purchased from Sigma Chemical Co. (St. Louis, MO). All other chemicals were of reagent grade.

Preparation of water extracts and 50% ethanol extracts of sufu The dressing mixture for ripening sufu was decanted and the solid portion of sufu was obtained. This solid portion was freeze-dried and then defatted using petroleum ether. Two hundred milligrams of freeze-dried and defatted sufu powder were suspended in 5 ml of distilled water or 5 ml of 50% (v/v) ethanol and kept at room temperature for 1 h. The suspension was centrifuged at 11,070 × g for 5 min at 4°C, and then the upper layer was filtered (0.5 μm).

Determination of radical-scavenging activity The radical-scavenging activities of the water and 50% ethanol

extracts of sufu were determined according to methods described by Suda (2000). Each extract (0.3 ml) was mixed with 0.2 M MES buffer (0.3 ml, pH 6.0) and ethanol (0.6 ml); 1.2 ml of a 400 μM DPPH solution in 0.1 M MES buffer (pH 6.0) containing 50% ethanol was then added. The mixture was kept at room temperature for 20 min, then the absorbance of the resulting solution was measured at 520 nm. The ability to scavenge the DPPH radical was expressed as the equivalent concentration of α-tocopherol (μg α-tocopherol/mg).

Determination of isoflavones in the extracts Isoflavones were analyzed quantitatively by high pressure liquid chromatography (HPLC) with a Dikma Diamonsil C_{18} column (ϕ4.6 × 250 mm) (Dima Co., Ltd., Orlando, FL). A linear gradient was employed where solvent A was 0.1% (v/v) acetic acid in water, and solvent B was 0.1% (v/v) acetic acid in acetonitrile. Solvent B increased from 15 to 35% in 50 min. The flow rate was 1.0 ml/min and the absorption was measured at 254 nm. The column temperature was 40°C. Quantitative data for daidzin, genistin, glycitin, daidzein, genistein, glycitein were obtained by comparison with known standards.

Results and Discussion

Radical-scavenging activity of sufu extracts Table 1 shows the production origin and color of the 14 sufu samples. The samples can be regarded as representative of the major types of sufu found in China. They were further classified according to color (red, white and grey sufu). Sufu color is based primarily on the different ingredients used in the dressing mixture during the ripening phase (Han *et al.*, 2001a).

Figure 1 shows the DPPH radical-scavenging activities of various sufu extracts. The radical-scavenging activity of the water extracts ranged from 2.03 to 11.93 μg α-tocopherol/mg, while those of the 50% ethanol extracts ranged from 2.14 to 14.62 μg α-tocopherol/mg; the activities of the latter extracts were thus higher than those of the former in all but one sample (sample No. 2).

Table 1. Origin and color of sufu samples.

Sample code	Origin of production	Color
1	North (HeiLongJiang)	Red
2	Middle (HeBei)	Red
3	Middle (BeiJing)	Red
4[a]	East (ZheJiang)	Red
5[a]	East (ZheJiang)	White
6	South (GuangDong)	White
7	South (GuangDong)	White
8	South-east (FuJian)	Grey
9	South-west (YunNan)	White
10	South-west (YunNan)	White
11[b]	South-west (GuangXi)	Red
12[b]	South-west (GuangXi)	White
13	South-west (GuiZhou)	Grey
14	West (SiChuan)	White

(a,b): Samples from the same manufacturer, respectively.

The grey sufu (samples No. 8 and No. 13) showed higher values of radical-scavenging activity in both the water and 50% ethanol extracts than that found for the red or white sufu extracts. No significant difference was found in radical-scavenging activity between the red and white sufu.

The samples from the same region showed similar activity (samples No. 6 and No. 7; No. 9 and No. 10). While the radical-scavenging activity of the extracts was influenced by producing region, there was no clear directional tendency (south or north, east or west) between the region of production and high radical-scavenging activity.

In our previous study (Wang *et al.*, 2003), peptides result-

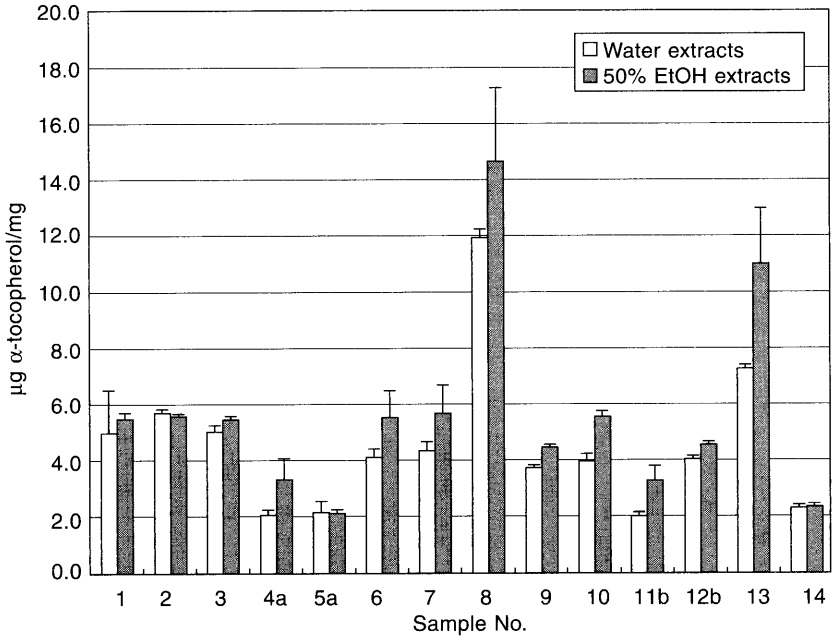

Fig. 1. Radical-scavenging activities of water extracts and 50% ethanol extracts of sufu from various regions. Sample No. 1 to 14, the same samples as in Table 1.

ing from the sufu fermentation were shown to exhibit the radical-scavenging activity. Isoflavones are known to have antioxidative activity, but it was reported that low DPPH radical-scavenging activity was observed in isoflavones (Suda *et al.*, 2003), and the activity of isoflavones was estimated as 1/15 of that of α-tocopherol (Hirota *et al.*, 2000). It was reported that 8-hydroxydaidein (8-OHD), 8-hydroxygenistein (8-OHG) and syringic acid in fermented soybean food had as high a DPPH radical scavenging activity as that of α-tocopherol (Hirota *et al.*, 2000). Therefore, the total radical-scavenging activity of the sufu samples may occur from the combined function of peptides, α-tocopherol, 8-OHD, 8-OHG and syringic acid. However, we need to carry out further experiments to determine the major material contributing to this radical-scavenging activity in sufu.

Isoflavone content and composition of sufu extracts

Table 2 shows the content and composition of isoflavones in water extracts of sufu. The isoflavone glucosides (daidzin, genistin, glycitin) were not detected in most of the samples. The content of the isoflavone aglycones (daidzein, genistein, glycitein), on the other hand, ranged from 50.2 to 179.3 μg/g of dry matter.

Table 3 shows the content and composition of isoflavones in 50% ethanol extracts of sufu. The isoflavone glucosides were detected in all but one sample (sample No. 7). Content of the isoflavone aglycones ranged from 199.0 to 706.9 μg/g of dry matter.

There was no clear directional tendency between the region of production and isoflavone content. One reason for this is that isoflavone content was greatly affected by the tofu preparation. The contents of isoflavone aglycones in the 50% ethanol extracts were higher than those of isoflavone glucosides in all the samples. It was reported that the isoflavone glucosides were hydrolysed into their corresponding aglycones during sufu fermentation (Li *et al.*, 2003). Our results also showed that content of isoflavone glucosides was very low in all the sufu.

In this study, significant variations in the radical-scav-

Table 2. Content and composition of isoflavones in water extracts of sufu.

Sample code	Glucoside (μg/g of defatted dry matter)				Aglycone (μg/g of defatted dry matter)			
	Daidzin	Glycitin	Genistin	Total	Daidzein	Glycitein	Genistein	Total
1	nd	nd	4.1	4.1	60.1	4.6	26.0	90.7
2	nd	nd	nd	nd	58.1	4.6	47.9	110.6
3	nd	nd	1.5	1.5	31.9	3.7	24.6	60.2
4[a]	nd	nd	nd	nd	58.7	3.9	46.6	109.2
5[a]	nd	nd	nd	nd	76.3	5.0	48.9	130.2
6	nd	nd	nd	nd	45.6	6.2	24.5	76.3
7	nd	nd	nd	nd	28.4	11.8	10.1	50.3
8	nd	nd	nd	nd	49.3	3.5	43.5	96.3
9	nd	nd	nd	nd	64.3	7.6	54.2	126.1
10	nd	nd	nd	nd	107.1	7.3	64.9	179.3
11[b]	nd	nd	nd	nd	58.8	3.7	38.8	101.3
12[b]	nd	nd	nd	nd	48.5	4.1	46.2	98.8
13	1.2	nd	nd	1.2	33.3	6.2	18.4	57.9
14	nd	nd	nd	nd	53.9	4.4	39.9	98.2

nd, not detected.
(a,b): Samples from the same manufacturer, respectively.

Table 3. Content and composition of isoflavones in 50% ethanol extracts of sufu.

Sample code	Glucoside (μg/g of defatted dry matter)				Aglycone (μg/g of defatted dry matter)			
	Daidzin	Glycitin	Genistin	Total	Daidzein	Glycitein	Genistein	Total
1	0.9	nd	15.8	16.7	235.8	18.5	102.1	356.4
2	3.2	nd	0.8	4.0	232.0	18.8	184.6	435.4
3	2.8	nd	1.1	3.9	129.3	14.3	94.9	238.5
4[a]	0.7	3.3	4.0	8.0	255.5	17.4	202.2	475.1
5[a]	1.9	0.6	7.3	9.8	304.3	20.3	194.8	519.4
6	0.7	nd	0.9	1.6	176.3	24.3	96.3	296.9
7	nd	nd	nd	nd	111.8	46.9	40.3	199.0
8	15.5	4.7	3.1	23.3	203.1	15.7	179.3	398.1
9	3.9	1.0	3.3	8.2	243.3	29.3	207.5	480.1
10	3.8	1.8	nd	5.6	419.1	29.9	257.9	706.9
11[b]	2.1	nd	1.9	4.0	230.5	15.1	151.7	397.4
12[b]	0.6	nd	nd	0.6	193.8	16.9	183.6	394.3
13	6.7	0.7	2.4	9.8	131.5	24.7	72.3	228.5
14	1.7	nd	nd	1.7	216.4	17.6	162.9	396.9

nd, not detected
(a,b): Samples from the same manufacturer, respectively.

enging activities of extracts from several types of sufu were observed. Variations in activity might be dependent on the conditions of processing including the fermentation. Li *et al.* (2003) reported that the change in isoflavone content and composition in sufu during the fermentation was significantly related to the salt content. Chiou and Cheng (2001) reported that isoflavone transformation during miso-fermentation was suppressed by the existence of ethanol and NaCl. Some kinds of peptides are produced through the enzymatic hydrolysis of soybean proteins by microorganisms. Alcohol, however, was found to delay the degradation of soybean proteins during the ripening stage (Chou and Hwan, 1994). These studies verified that low-salt and low-alcohol levels were beneficial to the increase in antioxidative activity during fermentation.

Among the three color types, red sufu is the most popular product in China because of its attractive color and strong flavor. In this research, however, it was found that the radical-scavenging activity of gray sufu was higher than that of red sufu. We are continuing our research using gray sufu and red sufu to identify the relationship between the physiological activity of sufu and its processing, e.g., type of microorganisms used, period of fermentation and the concentration of NaCl and ethanol. Through these attempts, we hope to establish improved processing methods for highly functional sufu that can increase the value of this substance.

Acknowledgement This study was conducted within the framework of the collaborative research project between Japan and China titled "Development of sustainable production and utilization of major food resources in China" supported by Japan International Research Center for Agricultural Sciences (JIRCAS).

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