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Antioxidative and Angiotensin I-Converting Enzyme Inhibitory Activities of Sufu (Fermented Tofu) Extracts

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Abstract

Tofuyo and sufu are fermented tofu products which are popular in Okinawa, Japan and in China, respectively. Water extracts from 4 types of tofuyo (produced in Okinawa, Japan) and one type of sufu (produced in Beijing, China) were prepared and their antioxidative activity and angiotensin I-converting enzyme (ACE) inhibitory activity were determined. The sufu extract showed higher antioxidative and ACE inhibitory activities than the 4 tofuyo extracts. There was a positive correlation between the antioxidative activity and ACE inhibitory activity of the extracts from the 5 samples. SDS-PAGE patterns of the extracts indicated that all the extracts mainly consisted of peptides whose molecular weights were less than 10 kDa. Although further investigations on the structure of the peptides in the extracts should be conducted and the relationship between the peptide structure and the 2 activities should be determined, it was shown that sufu contained highly active components and could be used as a functional food.

Discipline: Food
Additional key words: antioxidation, tofuyo

Introduction

Traditional fermented soybean foods have played an important role as nutritious foods in the diet of the people from olden times. There are many types of traditional fermented soybean foods such as miso, natto, and tempeh in the Asian countries. Sufu which is a fermented tofu product is popular all over China. There is a similar fermented tofu product in Okinawa, which is called tofuyo, and is considered to have originated in China. Although research on the processing and function of tofuyo has been carried out by the Yasuda group[10–14], the processing and function of sufu have not been systematically studied yet.

Recently, people have become increasingly interested in the physiological functionality of foods, in terms of antioxidative activity and anti-hypertensive effect. Many types of fermented soybean foods have been reported to exhibit a much stronger antioxidative activity than unfermented ones[3]. However, there are few investigations associated with such functions in sufu. Angiotensin I-converting enzyme (ACE) inhibitory activity in tofuyo was reported by Kuba and Yasuda[6], but the properties of sufu have not been fully documented.

The elucidation of the physiological functionality of sufu may enable to improve the fermentation process required to produce a highly functional food product in addition to the development of new products from sufu. In this study, we investigated the antioxidative activity and ACE inhibitory activity of sufu in China and of tofuyo in Okinawa.

Materials and methods

1. Materials

Samples from 4 types of tofuyo (sample 1 to sample 4) were purchased in Okinawa. A sample from one type

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of sufu (sample 5) was purchased in Beijing, China.

α, α-diphenyl-β-picrylhydrazyl (DPPH) and α-tocopherol were purchased from Wako Pure Chemical Co., Ltd. (Japan). Hippuryl-L-histidyl-L-leucine (Hip-His-Leu, HHL), angiotensin I-converting enzyme (ACE; from rabbit lung) and *o*-phthaldialdehyde (OPA) were purchased from Sigma Co., Ltd. (St. Louis, MO, USA). All the other chemicals were of reagent grade.

2. Preparation of water extracts from tofuyo and sufu

One hundred milligram of freeze-dried tofuyo or sufu powder was suspended in 2 mL distilled water and kept at room temperature for 30 min. The suspension was centrifuged at 11,070 × g for 5 min at 4ºC. The upper layer was filtered (0.5 μm).

3. Determination of antioxidative activity of the extracts

The effect of the water extracts from tofuyo or sufu on the DPPH radical was determined according to the method of Suda[9]. Each extract (0.3 mL) was mixed with 0.2M MES buffer (0.3 mL, pH 6.0) and ethanol (0.6 mL) to which 1.2 mL of a 400 μM DPPH solution in 0.1M MES buffer (pH 6.0) containing 50% ethanol was added subsequently. The mixture was kept at room temperature for 20 min, then the absorbance of the resulting solution was measured at 520 nm. α-tocopherol was used as a standard and the ability to scavenge the DPPH radical was expressed as the equivalent concentration of α-tocopherol (μg α-tocopherol/mg).

4. Determination of ACE inhibitory activity of the extracts

The ACE inhibitory activity was determined according to the method of Horie[4] as follows: 0.1 mL of the extract was mixed in a test tube with 0.1 mL of 0.025U/mL ACE and 0.1 mL of the substrate solution (4.7mM HHL and 600mM NaCl in 400mM phosphate buffer, pH 8.5). The mixture was incubated in a water bath at 37ºC for 30 min. Then 1.5 mL of 0.3M NaOH was added to terminate the reaction, and 0.1 mL of OPA solution (2% OPA in methanol) was added to the solution. After 20 min of incubation at room temperature, 0.2 mL of 3M HCl was added to terminate the derivative reaction, followed by 50-time dilution with distilled water. The fluorescence intensity was measured using a RF5300PC fluorescence spectrophotometer (Shimadzu Co., Ltd., Japan). The measurement conditions were as follows: excitation, 340 nm; emission, 455 nm; slit width, 10 nm. The reactions on the standard solutions and the blank were carried out by replacing the sample extracts and ACE solution with distilled water, respectively. The ACE activity was calculated from the following equation and expressed as ACE%:

$$ACE\% = (b - c) / a \times 100$$

where a is the fluorescence intensity of the standard solution (ACE), b is the fluorescence intensity of the reaction mixture with inhibitor (sample), c is the fluorescence intensity of the blank solution (without ACE). The ACE inhibitory activity was calculated based on the following equation:

$$\text{ACE inhibitory activity (\%)} = 100 - ACE\%$$

5. SDS-Polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was carried out using a PhastSystem (Amersham Pharmacia Biotech Co., Ltd.), on an 8–25% polyacrylamide gradient gel. Proteins were stained with the PhastGel Blue R. A LMW calibration kit (Amersham Pharmacia Biotech Co., Ltd.) was used for molecular weight standards.

Results

1. Antioxidative activities of tofuyo and sufu extracts

The antioxidative activities of the tofuyo and sufu extracts determined by the DPPH radical scavenging method are shown in Fig. 1. All the samples exhibited the antioxidative activity. Chinese sufu (sample 5) showed the highest antioxidative activity among the 5 samples, while in the Okinawa tofuyo samples, the antioxidative activity varied depending on the conditions of production.

2. ACE inhibitory activities of tofuyo and sufu extracts

The ACE inhibitory activities of the tofuyo and sufu

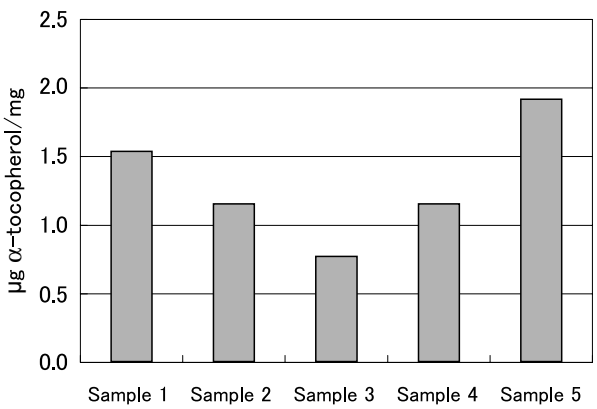

Fig. 1. Antioxidative activities of tofuyo and sufu extracts
Sample 1 to sample 4, various types of tofuyo from Okinawa, Japan; sample 5, sufu from China.

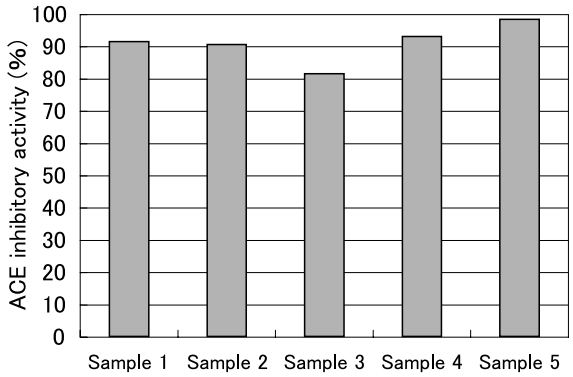

Fig. 2. ACE inhibitory activities of tofuyo and sufu extracts
Sample 1 to sample 4, various types of tofuyo from Okinawa, Japan; sample 5, sufu from China.

extracts are shown in Fig. 2. All the samples exhibited an ACE inhibitory activity, and sufu (sample 5) displayed the highest ACE inhibitory activity among the 5 samples. The 4 types of tofuyo showed different ACE inhibitory activities depending on the conditions of production. There was a positive correlation between the antioxidative activity and ACE inhibitory activity in the 5 samples.

3. SDS-PAGE patterns of tofuyo and sufu extracts

Molecular weight distribution of the proteins and peptides extracted from tofuyo or sufu was examined by SDS-PAGE. Fig. 3 shows the SDS-PAGE patterns of the tofuyo and sufu extracts. Bands of soybean β-conglycinin and glycinin were not detected in the patterns. The main components of the extracts were peptides with a molecular weight below 10 kDa. It was estimated that the samples with high antioxidative and ACE inhibitory activities contained a large amount of small peptides.

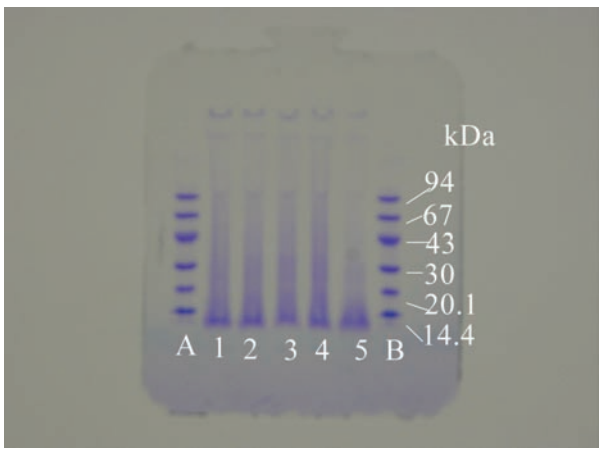

Fig. 3. SDS-PAGE patterns of tofuyo and sufu extracts
A and B, molecular weight standard; sample 1 to sample 4, various types of tofuyo from Okinawa, Japan; sample 5, sufu from China.

Discussion

Many types of peptides have been reported to show an antioxidative activity. Chen et al.[1] analyzed the structure of the antioxidative peptides from soybean β-conglycinin, and identified 5 types of oligopeptides with 6 to 16 amino acid residues. Therefore, in our study, the antioxidative activity of the water extracts was ascribed to the peptides derived from soybean proteins during the fermentation process.

There are several reports on the ACE inhibitory activity of peptides from soybean proteins. Kawamura[5] reported that 4 types of peptides with 6 to 7 amino acid residues derived from β-conglycinin and glycinin inhibited ACE. Sang et al.[8] also isolated ACE inhibitory peptides from a soybean hydrolysate. Fan et al.[2] hydrolyzed soybean protein using commercial proteases and prepared a hydrolysate with a high ACE inhibitory activity without bitterness. It was also reported that some physiologically active substances were produced and the activities were enhanced during fermentation. For example, some peptides were produced through enzymatic hydrolysis of soybean proteins by the action of microorganisms[7]. In our study, it was shown that the soybean proteins were hydrolyzed and functional peptides were derived during the fermentation process.

Yasuda et al.[11] reported the presence of changes in the chemical components of tofuyo prepared using *Monascus* fungus during fermentation. According to the report, protein digestion was the most conspicuous change during fermentation. At the beginning of fermentation, there were bands of major soybean proteins on the SDS-PAGE patterns, but all the bands, except for the basic subunit of glycinin, disappeared after 3 months of fermentation. Therefore, they concluded that the main components of tofuyo were the basic subunit of glycinin and peptides with a molecular weight of about 11–15 kDa. Similar changes in the proteins of sufu may occur during the fermentation process.

Significant variations in the antioxidative and the ACE inhibitory activities of the extracts from several types of Okinawa tofuyo and the extracts from Chinese sufu were observed. Such variations might be closely related to the conditions of processing, e.g. kind of microorganism used, duration of fermentation and so on. As a result of various conditions of fermentation, many kinds of peptides with different activities could be produced during the fermentation process.

The major components responsible for the antioxidative and the ACE inhibitory activities of the tofuyo or

sufu extracts were considered to be peptides. However, it is still possible that other components than peptides displayed these activities. Further studies should be conducted on the isolation and identification of the components in the extracts.

There are many types of sufu products in China. Microorganisms and fermentation conditions depend on the producing regions. We are continuing our studies on the identification of the physiological activity of various sufu products and on the analysis of the relationship between the function and the conditions of processing. Through these attempts, we may be able to develop an improved processing method for the production of highly active functional sufu to increase the demand for sufu.

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