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Development of New Extraction Method of Natural Antioxidants from Bamboo Grass

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The effect of the extraction method consisted of steam explosion followed by hot water and methanol extractions from *Sasa palmate* on the properties of extracted phenolic compounds were studied experimentally. The amounts of phenolic compounds in water extract and methanol extract from exploded *Sasa* leaf were 12 and 51 times larger than those from untreated *Sasa* leaf, respectively. The total amount of phenolic compounds extracted from exploded *Sasa* leaf was 175.3 (g/g-*Sasa*). The radical scavenging activity of methanol extract was as high as that of BHA at a high concentration of 100 mg/l. The antioxidant activity of methanol extract reached a maximum value of 89 %.

Key words: phenolic compound, Steam explosion, *Sasa palmata*, Radical scavenging activity, Antioxidant activity

1. INTRODUCTION

Active oxygen has strong oxidizability and reactivity. This strong oxidizability functions as the immune system that protect from disease –causing germs and harmful substance in vivo. However, the excessive active oxygen is hostile and damaging to cells and their functions. In living systems, dietary antioxidant compounds (phenolic compounds, Vitanin E, α-tocopherol, and ascorbic acid) and endogenous enzymes (superoxide dismutase, glutathione peroxidase and catalase) can protect the organism against oxidative damage. When the mechanisms of antioxidant protection become unbalanced by some factors, deterioration of physiological functions may result in degenerative or pathological processes such as aging, cancer, coronary heart diseases, etc [1]. However, the vitamins such as ascorbic acid and β-carotene in fruits and vegetables protect human body from oxidative damage by reacting with active oxygens and scavenging them. Epidemiological studies have shown that the consumption of fruits and vegetables is related with reduced risk of many diseases such as heart disease, cancer, arthritis and the aging process [1,2]. Consequently, the discovery of natural antioxidant compounds such as carotenoids, flavonoids, isoflavonoids, isothiocyanates and organosulfur compounds have been one of the major areas of recent scientific research [3-5]. Recently it is reported that the antioxidant compounds were abundantly included in the bamboo grass [6,7]. However, the effective extraction method of the antioxidant compounds from plant such as bamboo is not established. Meanwhile, it is reported that the steam explosion treatment was an effective pretreatment for separating and extracting various compoments from plant biomass [8].

In this work, the extraction and separation method of antioxidant compounds was investigated by using bamboo grass of *Sasa palmata*. In order to increase the amount of antioxidant compounds extracted, steam explosion treatment followed by hot water and methanol extractions was attempted, and then the optimal steam explosion condition such as steam temperature and steaming time was determined. In addition, the properties of the antioxidant compounds extracted were evaluated.

2. MATERIAL AND METHOD

2.1 Plant material

Sasa palmata collected in the forest area of Kanazawa University, Japan, was used as a plant material sample in this study. *S. palmata* leaf was air-dried to a constant weight immediately after collection and the cut into about 1 cm in length.

2.2 Steam explosion

Steam explosion apparatus (Japan Chemical Engineering and Machinery Co., Ltd, Osaka, Japan) consisted of a high pressurized reactor, a steam generator, a receiver, and a condenser with a silencing action [9,10]. The reactor was maintained at a constant temperature. The capacity of the reactor was 1.2 dm^3 and the highest temperature was 275 °C (5.5 MPa). 50 g of *S. palmata* leaf was put into the reactor and then steam-heated at a steam temperature of 180-260 °C (1.0-4.9 MPa) for a steaming time of 0.5-20 min. A ball valve at the bottom of the reactor was then suddenly opened to bring the reactor rapidly to the atmospheric pressure. The product containing solid and liquid materials was recovered in the receiver.

2.3 Extraction method

Extraction method for obtaining the antioxidant compounds from *Sasa palmata* consisted of two stages, i.e. hot water and methanol extractions, as shown in Fig. 1. *Sasa palmata* leaf exploded at 180-260 °C with 0.5-20 min and untreated *S. palmata* leaf are used as a sample. Initially, one gram of dry sample was extracted in a 300 ml erlenmeyer flask with 100 ml distilled water at 98 °C for 2 h. The resulting residue I was further extracted in a 300 ml erlenmeyer flask with 100 ml methanol at 25 °C for 2 h.

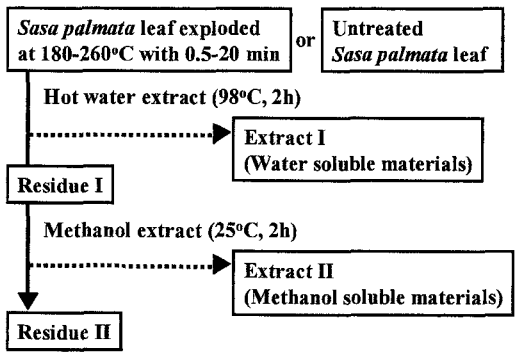

Fig.1 Extraction and separation methods.

2.4 Determination of phenolic compounds

The phenolic compounds in the extract were determined according to the Folin-Ciocalteu Method [11]. The extract (200 μl) was added to the test tube containing 4 ml of water, followed by addition of 1 ml phenol reagent (diluted five times with water). The mixture was thoroughly stirred. In addition, 1 ml of 10 % (w/v) sodium carbonate was added to this solution. After a 1 h incubation and at 30 °C, the increase in absorbance at 760 nm was measured. Estimations were carried out in triplicate and calculated from a calibration curve obtained with (+)-catechin. Phenolic compounds were expressed as a (+)-catechin equivalent (mg/g-sample).

2.5 Determination of radical scavenging activity

The radical scavenging activities of the extracts were calculated based on the change of absorbance due to the decrease in the stable 1,1-diphenyl-2-picrylhdrazyl free radical (DPPH radical) in relation to the control value [12,13]. DPPH radical is a stable nitorogen-centred free radical the color of which changes from violet to yellow upon reduction by either the process of hydrogen- or electron-donation [14]. Each dry extract in water or methanol (2 ml), ethanol (2 ml), and 0.5 mM DPPH radical ethanol solution (1 ml) were mixed in the test tube. After a 30 min incubation in darkness at 30 °C, the decrease in absorbance at 517 nm was measured. Considering the color of extract, the ethanol (1 ml) instead of 0.5 mM DPPH radical solution (1 ml) was used as a color blank. As a control, water or methanol (1 ml) was added instead of the extract. The evaluation of radical scavenging activity was calculated as follows:

$$\text{Radical scavenging activity (\%)} = [\ (A_{517control} - (A_{517sample} - A_{517blank})) / A_{517control}\ ]\times 100 \quad (1)$$

where $A_{517sample}$ is the absorbance of the extract and DPPH radical solution at 517 nm after a 30 min incubation, $A_{517control}$ is the absorbance of DPPH radical solution at 517 nm, $A_{517blank}$ is the absorbance of the color blank solution at 517 nm.

2.6 Determination of antioxidant activity

The antioxidant activity was determined by the conjugated diene method [15,16]. Each dry extract in methanol (100 μl) was mixed with 2 ml of 10 mM linoleric acid emulsion (pH 6.5) in test tubes. The test tubes were placed in darkness at 37 °C to accelerate oxidation. After a 15 h incubation, 6 ml of 60 % methanol in deionised water was added, and the absorbance of the mixture was measured at 234 nm against at blank. The evaluation of antioxidant activity was calculated as follows:

$$\text{Antioxidant activity (\%)} = [(A_{234control} - (A_{234sample} - A_{234blank})) / A_{234control}]\times 100] \quad (2)$$

where $A_{234sample}$ is the absorbance of the extract in methanol and the linoleric acid emulsion at 234 nm after a 15 h incubation, $A_{234control}$ is the absorbance of linoleric acid emulsion at 234 nm after a 15 h incubation, $A_{234blank}$ is the absorbance of the extract in methanol at 234 nm after a 15 h incubation.

3. RESULT AND DISCUSSION

3.1 Effect of steam explosion on extraction of phenolic compounds

Figure 2 shows the effect of steam temperature and steaming time on the amount of phenolic compounds in extract I and extract II. At steam temperature of 250 °C and steaming time of 1 min, the amounts of phenolic compounds contained in extract I and extract II reached a highest. The steam explosion of short time and high temperature was effective in the extraction of phenolic compounds.

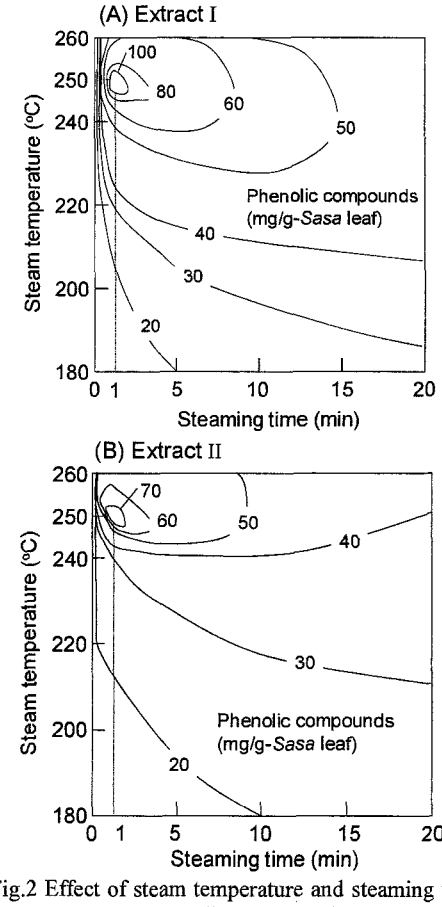

Fig.2 Effect of steam temperature and steaming time on the amounts of phenolic compounds in extract I and extract II.

3.2 Comparison of amounts of phenolic compounds from exploded *Sasa* leaf and untreated *Sasa* leaf.

Table 1 shows the comparison of amounts of phenolic compounds from exploded *Sasa* leaf and untreated *Sasa* leaf. Steam explosion conditions were steam temperature of 250 °C and steaming time of 1 min. The amount of phenolic compounds in extract I and extract II from exploded *Sasa* leaf were 12 times and 51 times more than those in extract I and extract II from untreated *Sasa* leaf, respectively. The amount of phenolic compounds was 18 times in total. Specifically, the amounts of phenolic compounds in extract I and extract II were 103.4 and 71.9 mg/g-*Sasa* leaf, respectively. This is because the constituent of *Sasa* leaf was low-molecularized by rapid decompression following steaming under high temperature and high pressure, and solubilized to water and methanol. In the test of properties of phenolic compounds, the extracts from *Sasa* leaf exploded at steam temperature of 250 °C and steaming time of 1 min were used.

Table 1 Comparison of amounts of phenolic compounds from exploded *Sasa* leaf and untreated *Sasa* leaf.

Sample	Phenolic compounds (mg/g-*Sasa* leaf)		
	Extract I	Extract II	Total
Untreated *Sasa* leaf	8.4	1.4	9.78
Exploded *Sasa* leaf *	103.4	71.9	175.3

*Steam temperature of 250°C and steaming time of 1 min

3.3 Radical scavenging activity

Figure 3 shows the radical scavenging activity of extract I, extract II and BHA. The radical scavenging activity of extract II was higher than that of extract I. This reflects that the phenolic compounds with high radical scavenging activity were included in extract II. The radical scavenging activity of low concentration of extract II was inferior to that of BHA. But the radical scavenging activity of 100 mg/l of extract II was as high as that of BHA.

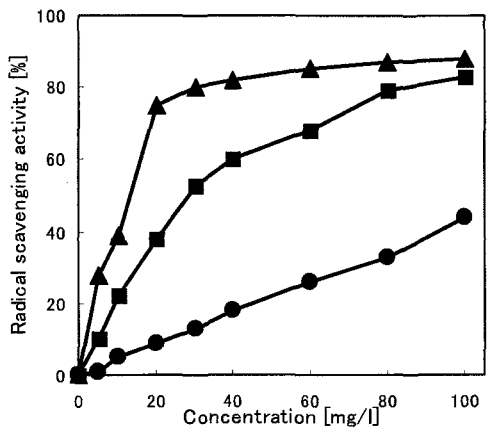

Fig.3 Radical scavenging activity of extract I, extract II and BHA. ●:Extract I, ■:extract II, ▲:BHA

3.4 Antioxidant activity

Figure 4 shows the antioxidant activity of extract I, extract II and BHA. The antioxidant activity of extract II was higher than that of extract I. The antioxidant activity of methanol extract reached 89 % in the maximum.

It was thought that pyrolysis products like levulinic acid were produced, and such degradation products may contribute to the antioxidant activity, in addition the phenolic compounds from lignin. It was suggested that the methanol extract had the possibility as health food or the food additive because it showed high antioxidant activity.

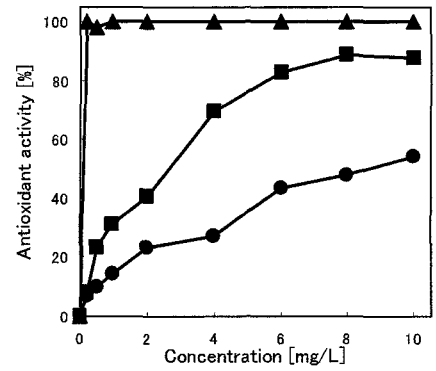

Fig.4 Antioxidant activity of extract I, extract II and BHA. ●:Extract I, ■:extract II, ▲:BHA

4. CONCLUSION

The steam explosion for 1 min at a steam temperature of 250 °C was a very effective pretreatment for the extraction of phenolic compounds from *Sasa* leaf. The maximum amount of phenolic compounds extracted from *Sasa* leaf was 175.3 mg/(g-*Sasa* leaf). The radical scavenging and antioxidant activities of methanol extract were higher than those of water extract. Furthermore, this extraction method composed steam explosion followed by hot water and methanol extractions will be applied to extraction of antioxidant compounds from not only *Sasa palmate* but also other plants.

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