NOTE

Antioxidant Characteristics of Extracts from Cereal Residues by Their Subcritical Water Treatment

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Abstract: Subcritical water treatment of cereal residues including okara, defatted rice bran, desalted soy sauce lees, sake lees, corn kernel hull, and defatted rapeseed was conducted at 260°C for 5 min to obtain the antioxidative extracts. The antioxidative activities of the extracts were evaluated using DPPH radical, peroxy radical, hydroxyl radical, hypochlorite ion, and peroxynitrite ion. The results show that the extracts from the sake lees, corn kernel hull, and defatted rapeseed had differently the antioxidative activities against all radicals and ions. However, the okara, defatted rice bran and desalted soy sauce lees had no activity against the hypochlorite ion.

Key words: cereal residues, antioxidative activities, subcritical water, 5-axe cobweb chart

1 INTRODUCTION

Cereal grains are well known as primary sources of naturally occurring antioxidants, such as phenolic compounds, isoflavones, phytates, and phytoestrogens, which contribute to reducing the risk of chronic diseases and promoting good health in humans. However, after food processing to obtain the desired products, a large quantity of cereal and grain residues with low values are produced and usually used for animal feed or discarded. It is highly possible that the cereal residues still contain a lot of the beneficial substances with antioxidant activities^{1,2)}.

The principal characteristic of an antioxidant is its competence to scavenge reactive radical species, such as reactive oxygen and nitrogen species, and make them unable to oxidize nucleic acids, protein, lipid or DNA in biological systems, which consequently initiate degenerative diseases in humans. In addition, the antioxidants may have ability to stop forming the reactive oxidants at the beginning of the radical generation. Thus, an antioxidant with a higher antioxidative activity can more effectively reduce free radicals in the body. The antioxidants may act as radical chain reaction inhibitors, metal chelators, oxidative enzyme inhibitors and antioxidant enzyme cofactors^{3,4)}.

To evaluate the activity of the specific antioxidants derived from foods, various methods have been used to measure and compare their activities. The activity depends on the specific radical or ion used as the reactant for the evaluation.

Subcritical water is hot water at a temperature between 100 and 374°C under high pressure in order to maintain its liquid state. The unique properties of subcritical water, which are advantageous in solid-liquid extraction processes, are its low dielectric constant and high ion product. The dielectric constant of subcritical water at about 250°C is as low as that of methanol and the ion product of the water is a few hundred higher orders in magnitude than that at ambient temperature. Consequently, subcritical water has ability to recover both polar and apolar substances from agricultural products. Our previous research has shown that the treatment of defatted rice bran at 260°C for 5 min provided the extracts with the highest DPPH scavenging activity^{5,6)}.

In this study, the antioxidant activities against DPPH, peroxy, and hydroxyl radicals, and the hypochlorite and peroxynitrite ions of the extracts from okara, defatted rice bran, desalted soy sauce lees, sake lees, corn kernel hull,

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and defatted rapeseed, which were treated with the subcritical water at 260°C for 5 min, were evaluated using the method of Terashima *et al.*⁷⁾.

2 EXPERIMENTAL

2.1 Materials

The peroxyxynitrite solution was obtained from Dojindo Laboratories (Kumamoto, Japan). Myoglobin from equine skeleton muscle was purchased from Sigma-Aldrich Japan (Tokyo, Japan). Iron sulfate heptahydrate, hydrogen peroxide, 2,2'-azobis-2-methyl-propanimidamide dihydrochloride (AAPH), 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium hypochlorite, and L-carnosine were purchased from Wako Pure Chemical Industries (Osaka, Japan). All the other chemicals and solvents were of analytical grade. Defatted rice bran was obtained from Tsuno Food Industrial (Wakayama, Japan). Soy sauce lees were obtained from Higashimaru Shoyu (Hyogo, Japan). Okara was obtained from Fuji Oil (Osaka, Japan). Japanese sake lees, defatted rapeseed and corn kernel hull were purchased from local markets in Kyoto, Japan.

2.2 Preparation of cereal residues

The soy sauce lees (200 g) were washed with deionized distilled water (500 mL) for 1 h to remove any residual salt. The sediment was collected using a filter paper (Toyo Roshi Kaisha, Tokyo, Japan). The washing process was repeated four times and the last filtrate was measured for residual sodium chloride using an electrical conductivity meter (CD-35MII, M&S Instrument, Tokyo, Japan) to verify that its concentration was lower than 0.1% (w/v). The residual water in the lees was removed using a freeze-drier (Eyela FDU-1200, Tokyo Rikakikai, Tokyo, Japan). The corn kernel hull was prepared by manually separating the corn seed and the hull, which was dried using the freeze-drier and ground using a mortar and pestle. All the other residues were used as received.

2.3 Subcritical water treatment of cereal residues

A cereal residue (140 mg) and deionized distilled water (7 mL) were placed in a 9-mL stainless steel vessel (Taiatsu Techno, Osaka, Japan). After sealing, the vessel was heated to 260°C and kept for 5 min using a temperature-controlled oven (GC-17A column oven, Shimadzu, Kyoto, Japan). It was then immediately cooled in an ice-water bath. The mixture inside the vessel was clarified by filtration through a 0.45 µm cellulose acetate membrane filter (Advantec Toyo, Tokyo, Japan). The clarified extract was dried using the freeze-drier and stored in a refrigerator until the analyses.

2.4 Measurement of antioxidant activities

The DPPH radical scavenging activity of the extracts was measured as described by Fujinami *et al.* (2001). The antioxidative activities of the extracts against the hypochlorite and peroxyxynitrite ions and the hydroxyl and peroxy radicals were measured using the method of Terashima *et al.* (2010), which were based on the structural changes in the myoglobin.

2.5 Statistical analysis

The DPPH radical scavenging activity and the myoglobin protective ratio of each extract were derived from triplicate measurements. The experimental data were evaluated for an analysis of variance (ANOVA), and the mean difference between the antioxidant activities of the extracts was done with Duncan's multiple range test at the significance level of $P < 0.05$ using the IBM SPSS statistics software (IBM Corporation, NY, USA).

3 RESULTS AND DISCUSSION

Figure 1 shows the radar charts of the antioxidant activities of the extracts from the okara, defatted rice bran, desalted soy sauce lees, sake lees, corn kernel hull, and defatted rapeseed against the five different reactive oxygen species. Each chart contains 5 axes including the DPPH radical scavenging activity and the antioxidative activities against the peroxy radical (AAPH), hydroxyl radical (OH), hypochlorite ion (CLO), and peroxyxynitrite ion (ONOO) expressed as the myoglobin protective ratios. The concentrations of the extracts were 20 g/L except for the corn kernel hull (10 g/L), which had higher activities than the others and exhibited 100% for the ONOO activity at 20 g/L.

Due to a rapid, simple and inexpensive method, the DPPH radical has been commonly used to compare the effectiveness of various antioxidants³⁾. As shown in Fig. 1, all the extracts from the various residues showed similar DPPH scavenging activities except for the extract from the corn kernel hull, which showed the highest activity at the same concentration as the others ($P < 0.05$). As a general trend, the free radical scavenging activity increased with the increasing extract concentration^{8,9)}. Because the DPPH radical does not exist in living systems, other reactive oxygen species, including the peroxy and hydroxyl radicals, and the hypochlorite and peroxyxynitrite ions, would also be used to evaluate the antioxidant activities of the extracts.

All the extracts could fully suppress the peroxy radical. The peroxy radical is generated from AAPH, which generally oxidizes lipids in cell membranes and subsequently generates lipid peroxides⁷⁾. Thus, the extracts from the cereal residues treated with the subcritical water may have protective effects on the cell membranes. It should be

Fig. 1 Radar charts of various extracts obtained by subcritical water treatment of (a) okara, (b) defatted rice bran, (c) desalted soy sauce lees, (d) sake lees, (e) corn kernel hull, and (f) defatted rapeseed. The concentration of each sample was 20 g/L except for the corn kernel hull (10 g/L). The labels, DPPH, AAPH, OH, CLO and ONOO, indicate 2,2-diphenyl-1-picrylhydrazyl radical, peroxy radical, hydroxyl radical, hypochlorite ion, and peroxynitrite ion, respectively. VC denotes L-ascorbic acid.

noted that the extracts from all the cereal residues showed high activities against the peroxy radical (100% of myoglobin protective ratio) at the extract to radical ratio of 1:10.

The extracts from various cereal residues showed different antioxidant activities against the hydroxyl radical, which was an extremely reactive and short-lived species that could hydroxylate DNA, proteins and lipid⁷⁾. The extracts from defatted rice bran, sake lees, and corn kernel hull could suppress the radical greater than 50% of the initial radical concentration, which agreed with Nagai's report¹⁰⁾. However, those from the desalted soy sauce lees and defatted rapeseed provided a low activity when compared to the others ($P < 0.05$). The high radical scavenging activity may be due to the antioxidants in the extracts acting as metal chelators and altering the activity of Fe(II)

in the tested solution. In contrast, some antioxidants may act as pro-oxidants and catalyze the hydroxyl radical generation, which caused the low activity⁴⁾.

Hypochlorite ion is a strong reactive oxygen species, which is, *in vivo*, generated by the reaction of H_2O_2 with chloride ions catalyzed by myeloperoxidase and reacts with sulfhydryl groups of the proteins in the living systems^{4, 7)}. Most of the extracts exhibited a low or no activity for scavenging the hypochlorite ion, but the one from the corn kernel hull provided a high activity at the same extract concentration as the others. Thus, the extract from the corn kernel hull is expected to have protective effects on the proteins.

The peroxynitrite ion is a kind of reactive oxidant. Compared to its protonated form, peroxynitrous (OONOH), the peroxynitrite ion is not a strong oxidant. Under physiological conditions, both the peroxynitrite and peroxynitrous forms can react with DNA, lipids, and proteins and trigger various oxidation reactions^{4, 11)}. The extracts from all the cereal residues provided a high activity ($> 70\%$ myoglobin protective ratio) against the peroxynitrite ion and the highest activity was derived from the corn kernel hull extract ($P < 0.05$).

The shape of the radar charts can assess the characteristics or quality of each antioxidant⁷⁾. Considering the activities against the peroxynitrite ion, and the DPPH and peroxy radicals, the extracts from all the cereal residues showed the similar antioxidant activities, while the extract from the corn kernel hull showed the highest activity, which was almost two times higher than those from the other residues at the same extract concentration. In addition, we divided the extracts into 2 groups according to the shapes of the radar chart. The first group with no activity against the hypochlorite ion included the extracts from okara, defatted rice bran and desalted soy sauce lees and the second one included the extracts from the sake lees, corn kernel hull and defatted rapeseed with different activities against all the 5 reactive radicals and ions. For the first group, the extracts from the desalted soy sauces lees showed the lowest activities against all the reactive radicals and ions. For the second one, the extracts from the defatted rapeseed and corn kernel hull exhibited the lowest and highest activities, respectively, against all the 5 reactive radicals and ions. Moreover, although the desalted soy sauce lees and okara were obtained from soybean, the extract from the former residue showed lower antioxidant activities than the latter one. The reason for this might be because more antioxidant substances in the soybean were transferred into the soy sauce during the fermentation process¹²⁾. Thus, the extracts obtained by the subcritical water treatment of the desalted soy sauce lees showed a lower antioxidant activity against the various reactive species.

The substances responsible for the antioxidant activity

may be obtained from phenolic compounds such as the ferulic, gallic, sinapic and vanillic acids and isoflavone^{5, 13–15}. Most of the phenolic compounds in cereal grains were normally bound to their cell wall and difficult to be recovered by traditional extraction methods, but the high temperature treatment by the subcritical water at 260°C could disintegrate the plant cell wall and release them as the free form into the extracts. In addition, the extracts contained not only the phenolic compounds, but also the other cell wall compositions, such as amino acids, peptides, proteins and carbohydrates⁵. Each component could react with each other to produce other substances including browning reaction products, which also contributed to the antioxidant activity of the extracts^{5, 16}.

The radar charts can reflect the different characteristics of the antioxidant activities of the cereal residues against the various free radicals. The extensive measurement using the shape of the radar charts including various kinds of reactive radicals could be used as a primary screening method to determine the difference in the physiological effects of each antioxidant for further development as a new food supplement from agricultural products and by-products.

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