Food Sci. Technol. Res., **10** (3), 264–267, 2004

Note

Radical-Scavenging Activity: Role of Antioxidative Vitamins in Some Fish Species

A. BHADRA[1], T. YAMAGUCHI[2], H. TAKAMURA[2,3] and T. MATOBA[2]*

[1]*Graduate School of Human Culture, Nara Women's University, Kitauoya-Nishimachi, Nara, 630-8506, Japan*
[2]*Department of Food Science and Nutrition, Nara Women's University, Kitauoya-Nishimachi, Nara, 630-8506, Japan*
[3]*Kyousei Science Center for Life and Nature, Nara Women's University, Kitauoya-Nishimachi, Nara, 630-8506, Japan*

Received October 30, 2003; Accepted April 14, 2004

To evaluate fish as free radical scavengers in the human diet, we examined the radical-scavenging activity of some marine fish tissue commonly consumed in Japan, using the 1,1-diphenyl-2-picrylhydrazyl (DPPH)-HPLC and 2′-deoxyguanosine oxidation method. All fishes showed high DPPH (259-2225 μmol Trolox eq./100g) and oxygen (1059-8018 μmol Trolox eq./100g) radical-scavenging activity. Ascorbic acid and tocopherol content were determined as free radical-scavenging compounds and ranged from 0.6–7.8 and 0.5–16.6 mg/100 g fresh weight, respectively. Fish liver and skin showed higher contents of these antioxidative compounds than other parts, although their contribution to DPPH radical scavenging activity was negligible. Unlike vegetables some other compounds seem to be responsible for the high scavenging activity of fish.

Keywords: radical-scavenging activity, fish, ascorbic acid, tocopherol.

Introduction

Reactive oxygen species are produced through normal metabolic processes *in vivo* and as a result of exposure to radiation or environmental pollution (Sardesai, 1995). These highly reactive species cause damage to cellular components including DNA and produce many degenerative diseases like cancer, heart disease, and diabetes. Living tissue can protect itself through different endogenous antioxidative processes. Besides internal defenses, the consumption of antioxidants from dietary sources can help in protecting against the oxidative stress and thus the onset of many chronic diseases.

Fish is integral part of the diet of people all over the world and the main source of protein in most Asian countries. Several epidemiological studies showed that consumption of fish lowers the risk of coronary heart disease and cardiovascular disease (Nestel, 2002; Rodriguez *et al.*, 1996; Kromhout, 1989; Kromhout *et al.*, 1985, 1995). Consumption of 30 g fish/day was found to be associated with 50% fewer deaths from coronary heart disease (Kromhout *et al.*, 1985). Coronary heart disease and cardiovascular disease are believed to be diseases which are induced by free radicals. Antioxidants are therefore apparently present in fish which are capable of neutralizing or scavenging free radicals. But the importance of fish in the diet is mainly considered to be for the presence of highly polyunsaturated fatty acids, especially n-3 fatty acids (Passi *et al.*, 2002; Ruiz de Gordoa *et al.*, 2002; Dyerberg *et al.*, 1975; Bang *et al.*, 1980). Mortality caused by cardiovascular disease was proved inversely proportional to the intake of n-3 fatty acids (Dolecek, 1992).

*To whom correspondence should be addressed.
E-mail: matoba@cc.nara-wu.ac.jp

The antioxidant defense system of fish was described by Filho (1996), Marcon and Filho (1999) and Ross *et al.* (2001). Hydrophilic, lipophilic and enzymatic antioxidants were quantified in some fish and shellfish by Passi *et al.* (2002). However, the contribution of antioxidative components in preventing free radical induced diseases has not be yet focused.

Though fish is consumed in large quantities in different nations, few reports are available on its antioxidative properties. Fish and fishery products showed high radical-scavenging activity comparable to vegetables in a recent study (Khanum *et al.*, 1999), but the active components remain to be identified. In this paper, we compared the antioxidant activity of different fish tissue using two different methods: the 1,1-diphenyl-2-picrylhydrazil (DPPH)-HPLC method developed by Yamaguchi *et al.* (1998) and the 2′-deoxyguanosine (2′-dG) oxidation method developed by Sakakibara *et al.* (2002). Antioxidative vitamins, ascorbic acid (AsA) and tocopherols and their contribution to radical-scavenging activity were also quantified the exploration for the active constituents responsible for this activity in fish.

Materials and Methods

Reagents DPPH, L-AsA, and 2,4-dinitrophenyl hydrazine were obtained from Nacalai Tesque Inc. (Kyoto, Japan). 2,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH), and 2′-dG were obtained from Wako Pure Chemical Industries (Osaka, Japan) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was obtained from Aldrich Chemical Co. (Milwaukee, WI, USA). Vitamin E reference standards (α-, β-, γ- and δ-tocopherols) and 2,2,5,7,8-pentamethyl-6-hydroxychroman (PMC) were obtained from Eizai, Ltd. (Tokyo, Japan).

Fish sample Four commonly consumed Japanese fishes representing three different groups, dark muscled (sardine), moderately dark muscled (horse mackerel) and white muscled (sea bream and flat fish) were selected for the experiment. All the samples were purchased in a chilled condition from local super markets and fish shops in Nara, Japan. Muscle, skin, and liver of each fish were excised carefully; then, each part was macerated/minced finely with a knife to ensure uniform sampling.

Identity of experimental fish Sardine (*Sardinops melanosticta*, length: 17.3 ± 1.5 cm, weight: 51.1 ± 14.6 g); horse mackerel (*Trachurus japonicus*, length: 19.8 ± 1.1 cm, weight: 66.7 ± 11.7 g); flat fish (*Limanda herzensteini*, 22.7 ± 1.8 cm, weight: 141.0 ± 35.3 g) and sea bream (*Chrysophrys major*, length: 25.0 ± 1.6 cm, weight: 277.3 ± 54.1 g).

DPPH radical-scavenging activity Preparation of sample extract: Minced fish sample (5–10 g) was homogenized for 50–60 s using a homogenizer (Kinematica Polytron homozinizer PT-MR2000) in 15–20 ml of water. The homogenate was centrifuged at 27000 × g for 20 min at 4°C. The supernatant was filtered through a 0.45–μm filter (Cosmonice Filter W, 13 mm, Nacalai Tesque Inc.), and the filtrate was designated as water extract. The residue was mixed with 15–20 ml chloroform/methanol (2:1, v/v) in a glass tube and was centrifuged at 1500 × g at 4°C for 20 min. Extraction was done twice. The resulting extracts were combined together and designated chloroform/methanol extract. This extract was dried in a rotary evaporator and dissolved again in the same volume of DMSO.

Radical-scavenging activity was measured according to the DPPH-HPLC method of Yamaguchi *et al.* (1998). The DPPH radical-scavenging activity was evaluated by the difference in peak area decrease of the DPPH radical between a blank and a sample. The activity was expressed as μmol Trolox equivalent per 100 g of fresh weight of fish sample. The total of the activities in both extracts was designated as the activity of each sample.

Oxygen radical-scavenging activity using 2′-dG oxidation method Sample extract was prepared in the same way as in the DPPH method. Suppression of 8-hydroperoxy-2′-deoxyguanosine (8-OOHdG) formation by the extracts was measured according to the method of Sakakibara *et al.* (2002). The radical-scavenging activity was evaluated by the difference in suppression of 8-OOHdG formation between a blank and a sample, and was expressed as μmol Trolox equivalent per 100 g of fresh weight of fish sample. The total of the activities in both extracts was designated as the activity of each sample.

AsA content Sample extract: Minced fish sample (5–10 g) was homogenized for 50–60 s using a homogenizer (Kinematica Polytron homogenizer PT-MR2000) in 15–20 ml of 5% metaphosphoric acid, and 5% metaphosphoric acid containing 1% stannous chloride solution. The resulting homogenate was centrifuged at 27000 × g for 20 min at 4°C, and the supernatant was filtered through a 0.45-μm filter (Cosmonice Filter W, 13 mm, Nacalai Tesque Inc.).

AsA content of fish parts was determined by HPLC following the method of Kishida *et al.* (1992). The content was calculated by subtracting the value of sample mixed with 2,6-dichloroindophenol from the value of sample without 2,6-dichloroindophenol. The data were expressed as mg per 100 g of each sample. To calculate the contribution of AsA to the radical-scavenging activity, AsA content was expressed as μmol of Trolox equivalent per 100 g of fresh fish sample.

Tocopherol content Tocopherol in fish samples was determined according to the method of Ueda and Igarashi (1990) with slight modification. Contribution of antioxidant vitamins to total radical-scavenging activity was calculated only for DPPH radical-scavenging activity because α-tocopherol was not antioxidative to oxygen radical (Sakakibara *et al.*, 2002), but AAPH produced oxygen radicals through an intermediary peroxyl radical.

Results and Discussion

DPPH radical-scavenging activity The DPPH radical-scavenging activity of horse mackerel, sardine, sea bream and flat fish is presented in Table 1. The results are in agreement with the previous work by Khanum *et al.* (1999), which reported the activity of the edible part (muscle and skin). Radical-scavenging activity of fish muscle is comparable to some vegetables such as burdock (490.0 μmol Trolox eq./100 g), broccoli (468.3), and eggplant (342.4) reported by Yamaguchi *et al.* (2001). Among the fish tested, horse mackerel and sardine muscle showed similar activity followed by sea bream and then flat fish. Fish liver and skin showed higher activity and liver activity was the highest. Among the four fishes, sardine liver was the highest in activity followed by horse mackerel, sea bream, and flat fish. Activity of fish skin was the highest for horse mackerel and lowest for flat fish. Activity of dark muscle was higher than ordinary muscle in horse mackerel but it was slightly lower in sardine. Dark muscle of horse mackerel showed higher activity than sardine. Flat fish muscle showed the lowest activity among all the fishes. Fish containing dark muscle showed overall higher activity compared to sea bream and flat fish.

Flat fish, a bottom dwelling and slow moving fish, showed lower activity than the other fishes. This result is consistent with the findings of Filho (1996) that more active marine fish possess enhanced antioxidant protection compared to sluggish fish according to their overall oxygen consumption. Janssens *et al.* (2000) observed that the level of enzymatic antioxidative defense is low in deep-sea fish.

Liver tissue usually had the highest level of antioxidants, which is closely related to fish activity level. Ross *et al.* (2001) found high antioxidant activity of fish liver and antioxidant levels were significantly higher in active fish than in those that were sedentary and sluggish (Filho, 1996).

Dark muscle of horse mackerel showed higher radical-scavenging activity than white muscle, which may be due to higher content of antioxidant in the former. Dark muscle of Atlantic mackerel (*Scomber scombrus*) showed higher content of lipid and lipid soluble antioxidants than the light muscle (Petillo *et al.*, 1998). Radical-scavenging activity

Table 1. DPPH radical-scavenging activity of different fish tissue.

	Horse Mackerel	Sardine	Sea bream	Flat fish
	(μmol Trolox eq./100g fresh tissue)			
Muscle without skin	476 ± 46[a]	499 ± 44	390 ± 45	303 ± 33
White muscle	382 ± 37	455 ± 33	NA[b]	NA
Dark muscle	521 ± 56	433 ± 34	NA	NA
Skin	1206 ± 66	572 ± 39	618 ± 129	259 ± 20
Liver	1361 ± 49	2225 ± 105	1111 ± 101	1028 ± 15

[a] All values are the means ± SD of three determinations.
[b] NA, not applicable

of horse mackerel skin was much higher than the other fish tested and the lowest value was found for flat fish skin. This may be related to the pigmentation of the skin.

Oxygen radical-scavenging activity Oxygen radical-scavenging activity of fish tissues measured using AAPH and 2′-dG is presented in Table 2. The activity of all the fish species was strong. Liver activity was 4 to 8 times higher than that of muscle activity, with sardine liver showed the highest. Dark muscle of horse mackerel and sardine showed higher activity than that of white muscle. Flat fish muscle showed the lowest activity among the four fish tested by the DPPH method, but it was comparable to the other fish when measured using the 2′-dG oxidation method. Skin of horse mackerel and sardine showed similar radical-scavenging activity and the values were nearly double sea bream and flat fish; the activity of these two species was similar for all tissues. The 2′-dG oxidation method was proved better for both water and lipid soluble antioxidants (Sakakibara *et al.*, 2002). Active constituents in fish seem to be highly soluble in water. Since AAPH produces molecular oxygen radical, the components present in fish can scavenge the oxygen radical more effectively than the DPPH radical. Difference in activity of the same tissue in different fish may be due to the presence of different active constituents and can be explained by individual feeding habits, species differences and maturity condition of individual species. As the oxygen radical-scavenging activity of fish is high, fish can be a rich source of antioxidants in a daily meal.

AsA Content AsA is a potent radical scavenger found in large quantities in fruits and vegetables, and it is the main contributor to the activity of some vegetables (Yamaguchi *et al.*, 2001). In the present study, the AsA and tocopherol content of fish was measured to understand the role of antioxidative vitamins in fish radical-scavenging activity. The results are shown in Table 3. AsA content of fish tissues was found low compared to that of fruits and vegetables. AsA content ranged from 0.6 to 7.8 mg/100 g fresh fish tissue and followed the same trend as DPPH radical-scavenging activity. Liver contained high AsA in all fish. Sardine liver showed the highest value followed by flat fish, sea bream, and horse mackerel. AsA contents of flat fish and sea bream muscle were similar and higher than that of horse mackerel and sardine muscle. Sardine skin showed the lowest AsA value among the four fish and was almost half that of the others. Since fish cannot synthesize AsA, the AsA value may vary with feeding habit, quality and availability of diet and season. Muscle AsA value for sardine and horse mackerel in the present study was almost two times higher than the findings of Passi *et al.* (2002). They found 2.3 μg/g and 2.5 μg/g for muscle tissue of sardine and horse mackerel, respectively. Hamre *et al.* (2003) observed variation in vitamin C and E content of herring from 1.0–3.3 mg/kg and 7–21 mg/kg fillets, respectively. Such variation in vitamin content may be primarily due to season, feeding condition and spawning condition of fish.

Contribution of AsA in DPPH radical-scavenging activity was low. Contribution of AsA to muscle activity of fish ranged 0.9–2.4%, skin activity 0.8–3.2% and liver activity 2.0–3.5% and in sea bream and flat fish muscle and skin was almost double that in horse mackerel and sardine (Table 3). Therefore, other antioxidative compounds are thought to play an important role in the radical-scavenging activity of these fish.

Tocopherol content All fish tissues were explored for α-, β-, γ- and δ-tocopherols, but only α-tocopherol was found in this study ranging 0.5–16.6 mg/100 g fresh tissue (Table 4). Fish liver showed the highest value followed by dark muscle and then skin and ordinary muscle. Sardine muscle showed the highest content followed by sea bream, horse mackerel and flat fish. α-Tocopherol content of flat fish liver was the highest among the fish tested, whereas muscle had the least. Sea bream skin showed twice the content of α-tocopherol than the other fish. Dark muscle of horse mackerel and sardine contained 4–6 times more α-tocopherol than white muscle in the present study. This result is similar to the findings of Petillo *et al.* (1998) for

Table 2. Oxygen radical-scavenging activity of different fish tissue.

	Horse Mackerel	Sardine	Sea bream	Flat fish
	(μmol Trolox eq./100 g fresh tissue)			
Muscle without skin	1372 ± 99[a]	1645 ± 86	1297 ± 184	1147 ± 206
White muscle	1059 ± 45	1333 ± 148	NA[b]	NA
Dark muscle	1839 ± 224	1572 ± 124	NA	NA
Skin	2164 ± 235	2189 ± 367	1197 ± 136	1488 ± 353
Liver	6253 ± 228	8018 ± 468	4098 ± 334	3981 ± 617

[a] All values are the means ± SD of three determinations.
[b] NA, not applicable

Table 3. AsA content of fish tissue.

	Horse Mackerel	Sardine	Sea bream	Flat fish
	(mg/100 g fresh tissue)			
Muscle without skin	0.6 ± 0.1[a] (1.0)[b]	0.7 ± 0.0 (0.9)	1.3 ± 0.2 (2.2)	1.2 ± 0.0 (2.4)
White muscle	0.6 ± 0.0 (1.0)	0.7 ± 0.1 (0.9)	NA[c]	NA
Dark muscle	1.0 ± 0.0 (1.3)	0.8 ± 0.0 (1.1)	NA	NA
Skin	1.7 ± 0.2 (0.9)	0.7 ± 0.2 (0.8)	1.5 ± 0.1 (1.5)	1.3 ± 0.0 (3.2)
Liver	4.3 ± 0.2 (2.0)	7.8 ± 0.3 (2.2)	5.0 ± 0.5 (2.8)	5.6 ± 0.0 (3.5)

[a] All values are the means ± SD of three determinations.
[b] Values in parenthesis are contribution to radical-scavenging activity (%) calculated from the activity of AsA (6.2 μmol Trolox eq./mg).
[c] NA, not applicable.

α-tocopherol in dark muscle of Atlantic mackerel, which was 3 times higher than that of light muscle. Passi *et al.* (2002) found 5.4 μg/g tocopherol for horse mackerel and 5.8 μg/g for sardine muscle. These values are also lower than our observed values. Liver α-tocopherol content of the fishes is comparatively higher than the findings of Filho (1996) for marine teleost (6.6 nmol/g) and that of fresh water migratory teleost (7.8 nmol/g) (Marcon and Filho, 1999).

The contribution of α-tocopherol to radical-scavenging activity in the present study was not high for the tissues and ranged from 0.2 to 3.4% (Table 4). The contribution to the dark muscle was higher than ordinary muscle, and even higher than liver except for flat fish. Below 1% contribution was observed to the activity of ordinary muscle, white

Table 4. α-Tocopherol content of fish tissue.

	Horse Mackerel	Sardine	Sea bream	Flat fish
	(mg/100 g fresh tissue)			
Muscle without skin	0.9 ± 0.0[a] (0.4)[b]	1.6 ± 0.1 (0.7)	1.3 ± 0.1 (0.7)	0.5 ± 0.0 (0.4)
White muscle	0.8 ± 0.1 (0.5)	0.7 ± 0.1 (0.3)	NA[c]	NA
Dark muscle	3.4 ± 0.2 (1.4)	4.6 ± 0.3 (2.2)	NA	NA
Skin	1.3 ± 0.1 (0.2)	1.2 ± 0.2 (0.4)	2.6 ± 0.2 (0.9)	1.0 ± 0.1 (0.7)
Liver	4.4 ± 0.4 (0.7)	6.5 ± 0.8 (0.6)	6.2 ± 0.6 (1.2)	16.6 ± 1.0 (3.4)

[a] All values are the means ± SD of three determinations.
[b] Values in parenthesis are contribution to radical-scavenging activity (%) calculated from the activity of α-tocopherol (2.2 μmol Trolox eq./mg).
[c] NA, not applicable

muscle and skin of the fishes. Though the contribution of AsA and α-tocopherol is negligible, the high radical-scavenging activity of fish liver can partly be explained by the presence of higher AsA and α-tocopherol content. The synergistic antioxidant effect of AsA and α-tocopherol is well established (Niki *et al.*, 1984; Leung *et al.*, 1981). Hiramoto *et al.* (2002) also found that some water-soluble antioxidants including AsA exhibited synergistic hydrogen donation activities when combined with tocopherol in a biphasic system. However, the contributions of other antioxidative compounds such as taurine, peptides, and carotenoids may also be important. Further studies on radical-scavenging compounds in fish are now in progress.

References

Bang, H.O., Dyerberg, J. and Sinclair, H.M. (1980). The composition of the Eskimo food in north western Greenland. *Am. J. Clin. Nutr.* **33**, 2657–2661.

Dolecek, T.A. (1992). Epidemiological evidence of relationships between dietary polyunsaturated fatty acids and mortality in the multiple risk factor intervention trial. *Proc. Soc. Exp. Biol. Med.* **200**, 177–182.

Dyerberg, J., Bang, H.O. and Hjorne, N. (1975). Fatty acid composition of the plasma lipids in Greenland Eskimos. *Am. J. Clin. Nutr.* **28**, 958–966.

Filho, D.W. (1996). Fish antioxidant defenses-a comparative approach. *Braz. Med. Biol. Res.* **29**, 1735–1742.

Hamre, K., Lie, O. and Sandnes, K. (2003). Seasonal development of nutrient composition, lipid oxidation and colour of fillets from Norwegian spring spawning herring (*Clupea harengus* L.). *Food Chem.* **82**, 441–446.

Hiramoto, K., Miura, Y., Ohnuki, G., Kato, T. and Kikugawa, K. (2002). Are water soluble natural antioxidants synergistic in combination with tocopherol? *J. Oleo Sci.* **51**, 569–576.

Janssens, J.B., Childress, J.J., Baguet, F. and Rees, J. (2000). Reduced enzymatic defense in deep-sea fish. *J. Exp. Biol.* **203**, 3717–3725.

Kishida, E., Nishimoto, Y. and Kojo, S. (1992). Specific determination of ascorbic acid with chemical derivatization and high performance liquid chromatography. *Anal. Chem.* **64**, 1505–1507.

Khanum, M.N., Yamaguchi, T., Hiroishi, S., Muraoka, F., Takamura, H. and Matoba, T. (1999). Radical-scavenging activities of fish and fishery products. *Food Sci. Technol. Res.* **5**, 193–199.

Kromhout, D. (1989). n-3 Fatty acids and coronary heart disease: epidemiology from Eskimos to Western populations. *J. Intern. Med.* **225**, 47–51.

Kromhout, D., Bosschieter, E.B. and De Coulander, L.C. (1985). The inverse relation between fish consumption and 20-year mortality from coronary heart disease. *N. Engl. J. Med.* **312**, 1205–1209.

Kromhout, D., Feskens, E.J.M. and Bowles, C.H. (1995). The protective effect of small amount of fish on coronary heart disease mortality in an elderly population. *Int. J. Epidemiol.* **24**, 340–345.

Leung, H.W., Vang, M.J. and Mavis, R.D. (1981). The cooperative interaction between vitamin E and vitamin C in suppression of peroxidation of membrane phospholipids. *Biochim. Biophys. Acta* **664**, 266–272.

Marcon, L.J. and Filho, D.W. (1999). Antioxidant process of the wild tambaqui, Colossoma macropomum (Osteichthyes, Serrasalmidae) from the Amazon. *Comp. Biochem. Physiol.* **123**, 257–263.

Nestel, P.J. (2002). Adulthood prevention: cardiovascular disease. *Med. J. Aust.* **176**, S118–119

Niki, E., Saito, T., Kawakami, A. and Kamiya, Y. (1984). Inhibition of oxidation of methyl linoleate in solution by vitamin E and vitamin C. *J. Biol. Chem.* **259**, 4177–4182.

Passi, S., Cataudella, S., De Marco, P., De Simone, F. and Rastrelli, L. (2002). Fatty acid composition and atioxidant levels in muscle tissue of different mediterranean marine species of fish and shellfish. *J. Agric. Food Chem.* **50**, 7314–7322.

Petillo, D., Hultin, H.O., Krzynowek, J. and Autio, J. (1998). Kinetics of antioxidant loss in mackerel light and dark muscle. *J. Agric. Food Chem.* **46**, 4128–4137.

Rodriguez, B.L., Sharp, D.S., Abbott, R.D., Burchfiel, C.M., Masaki, K., Chyou, P., Huang, B., Yano, K. and Curb, J.D. (1996). Fish intake may limit the increase in risk of coronary heart disease morbidity and mortality among heavy smokers. *Circulation* **94**, 952–956

Ross, W.S., Dalton, A.D., Kramer, S. and Christensen, B.L. (2001). Physiological (antioxidant) responses of estuarine fishes to variability in dissolved oxygen. *Comp. Biochem. Physiol.* **130**, 289–303.

Ruiz de Gordoa, J.C., de Renobales, M., del Cerro, A., Fernández de Labastida, E., Amiano, P., Dorronsoro, M. and the EPIC Group of Spain E. (2002). Habitual fish intake is associated with decreased LDL susceptibility to *ex vivo* oxidation. *Lipids* **37**, 333–341.

Sakakibara, H., Ashida, H. and Kanazawa, K. (2002). A novel method using 8-hydroperoxy-2′-deoxyguanosine formation for evaluating antioxidative potency. *Free Radical Res.* **36**, 307–316.

Sardesai, V.M. (1995). Role of antioxidants in health maintenance. *Nutr. Clin. Pract.* **10**, 19–25.

Ueda, T. and Igarashi, O. (1990). Determination of vitamin E in biological specimens and foods by HPLC-pretreatment of samples and extraction of tocopherols. *J. Micronutr. Anal.* **7**, 79–96.

Yamaguchi, T., Takamura, H., Matoba, T. and Terao, J. (1998). HPLC method for evaluation of the free radical-scavenging activity of foods by using 1,1-diphenyl-2-picrylhydrazyl. *Biosci. Biotechnol. Biochem.* **62**, 1201–1204.

Yamaguchi, T., Mizobuchi, T., Kajikawa, R., Kawashima, H., Miyabe, F., Terao, J., Kanazawa, K., Takamura, H. and Matoba, T. (2001). Radical scavenging activity of vegetables and the effect of cooking on their activity. *Food Sci. Technol. Res.* **7**, 250–257.