

## Radical-Scavenging Activities of Fish and Fishery Products

Mosammat Nazmanara KHANUM,<sup>1</sup> Tomoko YAMAGUCHI,<sup>1</sup> Sachiko HIROISHI,<sup>2</sup> Fumi MURAOKA,<sup>2</sup> Hitoshi TAKAMURA<sup>2</sup> and Teruyoshi MATOBA<sup>2,\*</sup>

<sup>1</sup>Graduate School of Human Culture, <sup>2</sup>Department of Food Science and Nutrition, Nara Women's University, Kitauoya-Nishimachi, Nara 630-8506, Japan

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**A total of 45 Japanese and Bangladeshi water fish and fishery products were investigated for radical-scavenging activity using a 1,1-diphenyl-2-picrylhydrazyl- HPLC method. Among the 35 Japanese fish and fishery products (37 items), cutlassfish showed the highest activity (565.7 mg Trolox eq/100 g) and seaweed showed the lowest (24.9 mg Trolox eq/100 g) on a fresh weight basis. Dried bonito, crab (abdomen), Pacific saury, horse mackerel, skipjack, halfbeak, tuna, sand borer, Pacific mackerel, barracuda and anglerfish showed activities of over 100 mg Trolox eq/100 g. The radical-scavenging activities of 10 Bangladeshi fish and fishery products varied from 37.9 to 202.1 mg Trolox eq/100 g. The stronger activity of cutlassfish was attributed to its silver colored skin. The active component was suggested to be uric acid, the metabolic end-product of guanine.**

Keywords: radical-scavenging activity, fish, fishery product, cutlassfish, uric acid

Free radicals generated from various phenomena such as environmental pollution, ultraviolet radiation, radiolysis and several normal metabolic processes *in vivo* including cellular respiration can cause serious oxidative damage to living cells and tissues. Reactive oxygen species rapidly interact with lipids, proteins and DNA molecules to induce membrane damage, denaturation of proteins, inactivation of enzymes, breakage of strand and base modification of DNA (Cross *et al.*, 1987). The consequences of such interactions are involved in the development of various disease states such as atherosclerosis, cardiovascular disease, cancer and various chronic diseases (Ames, 1989; Tappel, 1991).

Living tissues protect themselves from the oxidative damages of free radicals by not only the quenching and scavenging actions of such enzymes as superoxide dismutase, catalase, peroxidase, but also by low molecular weight compounds like tocopherol, phenolic compounds, ascorbic acid, *etc.* (Hodnick *et al.*, 1986; Niki, 1991). Besides endogenous defenses, consumption of dietary antioxidants play an important role in protecting against free radicals (Rimm *et al.*, 1993; Willett, 1994). Recently, naturally occurring antioxidants such as flavonoids and the related phenolics, which are present in foods and other biological materials, have been attracting the considerable interest of investigators. A correlation between the increased dietary levels of phenolic compounds and reduced coronary heart disease has been suggested, which may explain the protective effect of vegetable-rich diets on coronary heart disease (Aruoma *et al.*, 1993; Hertog *et al.*, 1993; Hertog, 1994). It is important to note that most of the investigations regarding the inhibitory effect of food components on the oxidative damages of biological

membranes have been devoted to the foods of plant origin. Much attention has been paid to the free radical-scavenging activity of fruits, vegetables, teas and various types of beverages (Wang *et al.*, 1996; Cao *et al.*, 1996).

Fish is an integral part of the human diet. Nearly 20 years ago, Bang *et al.* (1980) first suggested that the low mortality rate from coronary heart disease among Greenland Eskimos compared with Danes, may be due to their high consumption of seafood. Since that time, many other investigators have suggested that fish consumption has a protective effect against cardiovascular diseases (Norell *et al.*, 1986; Shekelle & Stamler, 1993; Kromhout *et al.*, 1985, 1995). However, the controversy surrounding the association between fish consumption and coronary heart disease arose from a few negative results (Ascherio *et al.*, 1995; Morris *et al.*, 1995) and inconsistent findings from several studies (Siscovick *et al.*, 1995; Daviglus *et al.*, 1997). Recently, Albert *et al.* (1998) reported that consumption of fish at least once a week can cut the risk of sudden cardiac death in half. An inverse relationship between the consumption of n-3 polyunsaturated fatty acids and sudden cardiac death has also been reported (Norell *et al.*, 1986). The n-3 fatty acids are important components of all cell membranes, which have been demonstrated to have anti-inflammatory effects, and may have beneficial effects on ulcerative colitis, rheumatoid, arthritis, and asthma (Simopoulos, 1991; Broughton *et al.*, 1997). Epidemiological studies suggest that fish consumption and n-3 fatty acids may have beneficial effects on certain types of cancers (Mishina *et al.*, 1985; Willett *et al.*, 1990). However, the protective effects of other constituents present in fish have yet to be explored. Since cardiovascular disease (Kushi *et al.*, 1995), cancer (Hertog *et al.*, 1995), inflammation (Middleton & Kandaswami, 1992) *etc.* are considered as free radical-induced diseases, antioxidants which are capable of neutralizing or scavenging free radicals may therefore be of central

\* To whom correspondence should be addressed.

Abbreviations: Tris, tris(hydroxymethylamino)methane; AMP, adenosine 5'-monophosphate; GMP, guanosine 5'-monophosphate; IMP, inosine 5'-monophosphate; DPPH, 1,1-diphenyl-2-picrylhydrazyl.

importance in the prevention of these diseases.

Therefore, it is very important to assess the antioxidant activity of fish and fishery products to obtain a better understanding of their protective effect against free radical-induced diseases. In this study, the radical scavenging activities of 45 fish and fishery products (including a few processed fish products) were investigated. We focused on 35 Japanese fish and fishery products commonly consumed by Japanese people and 10 Bangladeshi fish and fishery products commonly consumed by Bangladeshi people. We have determined the free radical-scavenging activity as a measure of the antioxidant properties of various fish and fishery products.

## Materials and Methods

**Materials** Japanese fish and fishery product samples were purchased from local supermarkets in Nara in either fresh, boiled, or frozen condition. The samples were collected regularly and studied as soon as possible. Bangladeshi fish were purchased in frozen condition from a Bangladeshi shop (Shonali Trade International) in Tokyo. The fish were stored at  $-50^{\circ}\text{C}$  until evaluated.

Tris(hydroxymethylamino)methane (Tris) was obtained from Aldrich Chemical Co. (Milwaukee, WI). Adenosine 5'-monophosphate (AMP), guanosine 5'-monophosphate (GMP), inosine 5'-monophosphate (IMP), 1,1-diphenyl-2-picrylhydrazyl (DPPH), and uricase (4.95 U/mg) were purchased from Nacalai Tesque Inc. (Kyoto). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), hypoxanthine, guanosine, guanine, and ethanol and methanol of HPLC-grade were obtained from Wako Pure Chemical Industries (Osaka). Distilled water purified by Milli-Q Labo (Millipore, Tokyo) was used throughout the experiment.

**Determination of moisture content** Before measuring the radical-scavenging activity, moisture content of all the samples was determined according to the standard method of the Association of Official Analytical Chemists (1970).

**Preparation of sample extract** To obtain a water-soluble extract, 10 g of an edible part, which includes muscle and skin, was excised from each fish and homogenized with a small amount of water by a homogenizer (Nissei AM-8 homogenizer). The resulting homogenate was diluted with ultra pure water and the volume was adjusted to 50 ml. The obtained solution was then centrifuged at  $3000\times g$  for 20 min at  $4^{\circ}\text{C}$  and the supernatant was further centrifuged at  $15,000\times g$  for 20 min at  $4^{\circ}\text{C}$ . The supernatant finally obtained was filtered through a micro-filter (Cosmonice W,  $0.45\ \mu\text{m}$ , 13 mm i.d.) and the filtrate was used as the water-soluble extract of the sample.

To obtain an ethanol extract, 30 ml of ethanol was added to the residual sample after water extraction, and shaken for 1 h with a mechanical shaker. The resulting suspension was then centrifuged at  $3000\times g$  for 20 min at  $4^{\circ}\text{C}$ . The supernatant obtained was designated as extract 1. Another 20 ml of ethanol was added to the remaining residual sample, and extracted again for 1 h with a mechanical shaker. The resulting suspension was then centrifuged at  $15,000\times g$  for 20 min at  $4^{\circ}\text{C}$ , and the supernatant obtained was designated as extract 2. Extracts 1 and 2 were then combined and filtered through a micro-filter. The filtrate was used as the ethanol-

soluble extract of the sample.

**Measurement of the radical-scavenging activity** Radical-scavenging activities of water and ethanol-soluble extracts were assayed according to the DPPH-HPLC method of Yamaguchi *et al.* (1998a). The sample extract ( $200\ \mu\text{l}$ ) was incubated in 0.5 mM DPPH-methanol solution (1 ml) and 100 mM Tris-HCl buffer ( $800\ \mu\text{l}$ ), pH 7.4 for 20 min at room temperature in the dark. The reaction mixture was then subjected to a reversed phase HPLC analysis. The analysis was carried out using a TSK-GEL Octyl-80 TS ( $4.6\times 150\ \text{mm}$ ) column equipped with a Shimadzu SPD-10AV UV-VIS detector at 517 nm at room temperature. Methanol/water (70:30, v/v) was used as the mobile phase at a flow rate of 1 ml/min. The Tris-HCl buffer (1 ml) incubated in the DPPH solution (1 ml) was analyzed as a control. Trolox, a stable antioxidant, was used as a standard and  $200\ \mu\text{l}$  of ethanol-Trolox solution ( $50\ \mu\text{M}$ ) was assayed similarly during each run. Radical-scavenging activity was calculated from the following equation and expressed as mg Trolox eq/100 g of the edible part:

$$\text{mg Trolox eq} = \frac{(A-B)/(A-C) \times 5 \times 2 / 1000 \times D / 0.2}{\times 100 / 10 \times 250.29 / 1000}$$

where *A*: peak area of control, *B*: peak area of sample, *C*: peak area of Trolox, *D*: dilution factor, 250.29: molecular weight of Trolox

The combined activities of the water-soluble and ethanol-soluble extracts were used as the total activity of an individual sample. All the data are presented as the mean value of three determinations.

**Effect of uricase enzyme on the radical-scavenging activity of cutlassfish** The water soluble extract of cutlassfish ( $200\ \mu\text{l}$ ) was incubated in  $100\ \mu\text{l}$  uricase solution (0.2 mg/ml of 50 mM phosphate buffer, pH 7.8) for a period of 1, 10, 20, 40 and 60 min at  $20^{\circ}\text{C}$ . An aliquot of the incubated sample ( $200\ \mu\text{l}$ ) was analyzed to determine the radical-scavenging activity. The phosphate buffer solution ( $100\ \mu\text{l}$ ) was used as a blank in the absence of the enzyme solution.

## Results and Discussion

The identities of the different fish and fishery product specimens used in this study are presented in Table 1.

**Radical-scavenging activities of Japanese fish and fishery products** Table 2 shows the radical-scavenging activities of the 35 Japanese samples. There are 37 items in Table 2, since young and adult yellowtail were regarded as different items and abdomen and legs of crab were separately analyzed. The activities of the various fish and fishery products varied from 24.9 to 565.7 mg Trolox eq/100 g of edible part on a fresh weight basis. The highest activity was observed in cutlassfish and the lowest was found in seaweed. Dried bonito, red crab (abdomen), Pacific saury, horse mackerel, skipjack, halfbeak, tuna, sand borer, Pacific mackerel, barracuda and angler fish showed activities of over 100 mg Trolox eq/100 g of the edible part (fresh weight basis). Others showed activities below 100 mg Trolox eq/100 g of the edible part on a fresh weight basis. Among the raw fishes, kelp bass showed the lowest activity (36.0 mg Trolox eq/100 g of edible part). The radical-scavenging activity of

**Table 1.** Identities of different specimens used in the investigation.

Japanese			Bangladeshi		
Japanese name	English name	Scientific name	Bengali name	English name	Scientific name
<i>Aji</i>	Horse mackerel	<i>Trachurus japonicus</i>	<i>Aor</i>	Riverine catfish (1)	<i>Mystas aor</i>
<i>Ankou</i>	Anglerfish	<i>Lophiomus setigerus</i>	<i>Boal</i>	Freshwater shark	<i>Wallago attu</i>
<i>Asari</i> <sup>(a)</sup>	Short-necked clam	<i>Tapes japonica</i>	<i>Chital</i>	Featherback	<i>Notopterus chitala</i>
<i>Ebi (Taishoebi)*, (b)</i>	Shrimp	<i>Penaeus orientalis</i>	<i>Ilish</i>	River shad	<i>Hilsha ilisha</i>
<i>Hamachi, Buri</i>	Yellowtail (young, adult)	<i>Seriola quinqueradiata</i>	<i>Pangus</i>	Riverine catfish (2)	<i>Pangasias pangasias</i>
<i>Hamaguri</i> <sup>(a)</sup>	Hard Clam	<i>Meretrix lusoria</i>	<i>Puti</i>	Indian minor carp	<i>Barbus stigma</i>
<i>Hamo*</i>	Pike conger	<i>Muraenesox cinereus</i>	<i>Rui</i>	Indian common carp	<i>Labeo rohita</i>
<i>Hirame</i>	Japanese flounder	<i>Paralichthys olivaceus</i>	<i>Sharputi</i>	Indian major carp	<i>Burbus sarana</i>
<i>Hotategai</i> <sup>(a)</sup>	Scallop	<i>Patinoptecten yessoensis</i>	<i>Shol</i>	Snake headed fish	<i>Chana striatus</i>
<i>Ika (Surumeika)</i>	Squid	<i>Ommastrephes sloani pacificus</i>	<i>Chapa</i>	Semi-fermented Indian	
<i>Iwashi</i>	Japanese pilchard	<i>Sardinops melanostica</i>	<i>shutki</i> <sup>(d)</sup>	minor carp	
<i>Kaki</i> <sup>(a)</sup>	Oyster	<i>Crassostrea gigas</i>			
<i>Kamasu</i>	Barracuda	<i>Sphyrna schlegeli</i>			
<i>Kani</i>	Crab	<i>Chionoecetes japonicus</i>			
<i>(Benzuwaigani)**, (c)</i>					
<i>Karei</i>	Flatfish	<i>Limanda herzensteini</i>			
<i>Katsuo*, (c)</i>	Skipjack	<i>Katsuwonus pelamis</i>			
<i>Kawahagi</i>	Filefish	<i>Stephanolepis cirrhifer</i>			
<i>Kisu</i>	Sandborer	<i>Sillago japonica</i>			
<i>Kue*</i>	Kelp bass	<i>Stereolepis chinagi</i>			
<i>Maguro*</i>	Tuna	<i>Thunnus thynnus</i>			
<i>Managatsuo*</i>	Butterfish	<i>Pampus argenteus</i>			
<i>Saba</i>	Pacific mackerel	<i>Scomber japonicus</i>			
<i>Sake (Shirozake)*</i>	Salmon	<i>Oncorhynchus kata</i>			
<i>Samma</i>	Pacific saury	<i>Cololabis saira</i>			
<i>Sawara</i>	Spanish mackerel	<i>Scomberomorus niphonius</i>			
<i>Sayori</i>	Halfbeak	<i>Hemiramphus sajori</i>			
<i>Tachiuo</i>	Cutlassfish	<i>Trichiurus lepturus</i>			
<i>Tai (Madai)</i>	Sea bream	<i>Chrysophrys major</i>			
<i>Tako (Madako)**</i>	Octopus	<i>Octopus vulgare</i>			
<i>Tara</i>	Atlantic cod	<i>Gadus macrocephalus</i>			
<i>Wakame</i> <sup>(d)</sup>	Seaweed	<i>Underia pinnatifida</i>			
<i>Chirimenjako</i> <sup>(d)</sup>	Small dried sardine				
<i>Katsubushi</i> <sup>(d)</sup>	Dried bonito				
<i>Niboshi</i> <sup>(d)</sup>	Tiny dried sardine				
<i>Surume</i> <sup>(d)</sup>	Dried squid				

Japanese fish samples were purchased in a raw condition unless otherwise specified. Bangladeshi fish samples were purchased in a frozen condition. Edible parts including muscle and skin and excluding head, gut, and bone were used for analysis unless otherwise specified. \*Frozen, \*\*Boiled, <sup>(a)</sup>Whole body excluding shell, <sup>(b)</sup>Whole body excluding head and shell, <sup>(c)</sup>Muscle only, <sup>(d)</sup>Whole.

mollusk ranged from 36.4 (short-necked clam) to 49.9 (hard clam) mg Trolox eq/100 g on a fresh weight basis. Among the four processed products, dried bonito showed the highest activity (182.3 mg Trolox eq/100 g) followed by dried small sardine (56.2 mg Trolox eq/100 g) and tiny sardine (48.6 mg Trolox eq/100 g). On a moisture-free basis, the order of activity for the investigated fish species was similar to that obtained on a fresh weight basis (Table 2), which indicated that these species possess species-specific activities.

**Radical-scavenging activities of Bangladeshi fish and fishery products** Table 3 shows the radical-scavenging activities of 10 fish and fishery products in Bangladesh. The activity of raw Bangladeshi fish varied from 84.7 to 135.3 mg Trolox eq/100 g on a fresh weight basis. The highest activity was observed in featherback (135.3 mg Trolox eq/100 g) and the lowest value was found in riverine catfish (37.9 mg Trolox eq/100 g). The only processed fish (*chapa shutki*) prepared by the semi-fermentation of Indian minor carp showed an activity of 202.1 mg Trolox eq/100 g of the edible part. It should be noted that the Bangladeshi fish in this study were

purchased in a frozen condition, and therefore the effect of freezing and thawing on their activities cannot be excluded. However, the radical-scavenging activities of all the Bangladeshi fish were considerably high and the values were comparable to those of Japanese fish.

**Groupwise radical-scavenging activities of investigated fish species** All the fish species and invertebrates investigated in this study were divided into four categories: white muscle fish, red muscle fish, crustacean (crabs and shrimps) and mollusks. Figure 1 shows the radical-scavenging activities of these four categories. White muscle fish showed various levels of radical-scavenging activity, with the activity of cutlassfish remarkably higher than others. Red muscle fish and crustaceans (shrimps and crabs) also exhibited a higher level of radical-scavenging activity, while mollusks showed the lowest level.

**Comparison of the radical-scavenging activities of fish and vegetables** Vegetables are well known to contain compounds that protect against various diseases (Ames, 1983; Doll, 1990; Ascherio *et al.*, 1992). These compounds are

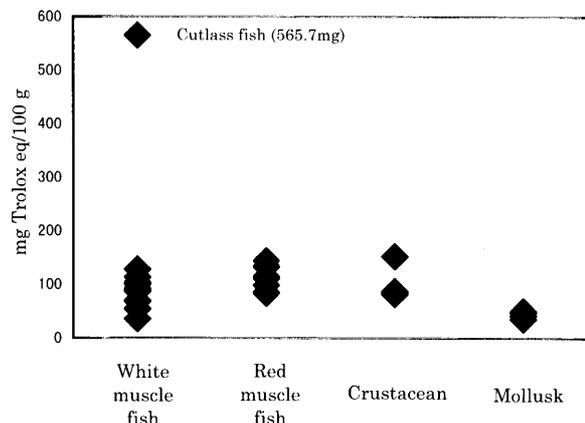
ascorbic acid, tocopherol, flavonoids, *etc.*, which have been found to possess antioxidant and free radical-scavenging activity in foods (Bors & Saran, 1987; Hertog *et al.*, 1993;

Mehra *et al.*, 1995; Cao *et al.*, 1996). Cabbage and Chinese cabbage have been reported to show antioxidant activity against hydroxyl (OH<sup>•</sup>) and peroxy (ROO<sup>•</sup>) radicals (Cao *et al.*, 1996). These vegetables also showed a high activity (75.0 and 25.0 mg Trolox eq/100 g, respectively, on a fresh weight basis) against the DPPH-radical (Yamaguchi *et al.*, 1998b). However, almost all the fish species in this study exhibited higher activity (Tables 1 and 2) than those of the two vegetables.

*The radical-scavenging activity of cutlassfish* To determine the effect of freshness on the radical-scavenging activity, cutlassfish was purchased from a local supermarket in Nara and two department stores in Nara (department store A) and Osaka (department store B), and analyzed accordingly. Two samples purchased from the supermarket and department store A were frozen and sliced while the other fish collected from department store B was kept fresh and intact. The activities of the three samples varied widely (Table 4). The

**Table 2.** Radical scavenging activity of Japanese fish and fishery products.

Item	Radical scavenging activity (mg Trolox eq/100 g)			
	Fresh weight basis			Dry weight basis
	Water extract	Ethanol extract	Total	Total
Cutlassfish	485.6	80.1	565.7	1654.0
Dried bonito	111.6	70.7	182.3	221.2
Crab (abdomen)	143.5	9.3	152.8	601.6
Pacific saury	137.8	6.7	144.5	377.7
Horse mackerel	123.9	10.7	134.6	606.3
Skipjack	132.4	0.5	132.9	474.6
Halfbeak	102.2	26.2	128.4	514.9
Tuna	113.5	1.7	115.2	438.1
Sandborer	104.9	8.5	113.4	550.6
Pacific mackerel	107.3	4.2	111.5	370.4
Barracuda	85.2	18.4	103.6	464.8
Anglerfish	90.8	9.7	100.5	616.5
Yellowtail (young)	96.4	2.4	98.8	254.0
Filefish	84.7	7.4	92.1	445.1
Japanese flounder	84.7	5.1	89.8	408.2
Salmon	84.2	5.4	89.6	291.9
Sea bream	75.2	14.3	89.5	311.5
Shrimp	75.5	12.2	87.7	419.5
Yellowtail (adult)	80.1	7.0	87.1	215.6
Spanish mackerel	75.8	10.4	86.2	274.5
Crab (leg)	71.3	13.1	84.4	353.5
Japanese pilchard	76.8	7.2	84.0	237.2
Octopus	65.2	17.4	82.6	384.7
Flatfish	64.0	6.2	70.2	303.8
Pike conger	56.8	11.7	68.5	200.9
Dried squid	50.0	13.3	63.3	81.1
Tiny dried sardine	42.6	13.6	56.2	63.2
Atlantic Cod	49.8	5.4	55.2	314.4
Butterfish	50.5	3.9	54.4	248.2
Squid	44.9	6.0	50.9	297.7
Hard clam	39.6	10.3	49.9	380.8
Small dried sardine	30.9	17.7	48.6	305.6
Oyster	42.4	7.0	49.4	73.9
Short-necked clam	39.7	3.1	42.8	233.9
Scallop	33.5	2.9	36.4	180.1
Kelp bass	35.2	0.8	36.0	103.7
Seaweed	13.0	11.9	24.9	497.6



**Fig. 1.** Groupwise radical scavenging activity of investigated fish species. Total activity on a fresh weight basis was used for groupwise classification.

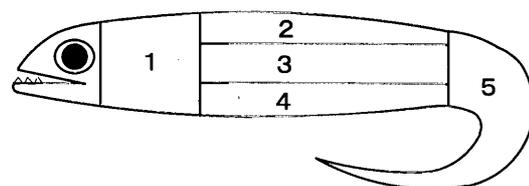
**Table 4.** Variation in the radical scavenging activity of cutlassfish purchased from different markets.

Place of purchase	Condition	Radical scavenging activity (mg Trolox eq/100 g)		
		Water extract	Ethanol extract	Total
Supermarket	Frozen, sliced	485.6	80.1	565.7
Department A	Frozen, sliced	212.9	54.2	267.1
Department B	Fresh, intact	162.6	41.4	204.0

The data is presented on a fresh weight basis.

**Table 3.** Radical scavenging activity of Bangladesh fish.

Item	Radical scavenging activity (mg Trolox eq/100 g)			
	Fresh weight basis			Dry weight basis
	Water extract	Ethanol extract	Total	Total
Semi-fermented	118.2	83.9	202.1	310.9
Indian minor carp				
Featherback	122.7	12.6	135.3	541.3
Indian common carp	108.5	15.3	123.8	531.3
Riverine catfish (1)	64.4	43.9	108.3	494.7
Indian major carp	81.3	23.4	104.7	351.2
Indian minor carp	72.9	14.4	87.3	305.1
Snake headed fish	71.5	13.2	84.7	385.0
River shad	59.2	24.7	83.8	181.1
Fresh water shark	46.7	12.8	59.5	220.4
Riverine catfish (2)	27.6	10.3	37.9	136.9



**Fig. 2.** Different parts of cutlassfish. 1, head; 2, back; 3, middle; 4, abdomen; 5, tail.

sample purchased from the supermarket had the highest activity (565.7 mg Trolox eq/100 g of fresh sample), while those purchased from department stores A and B showed activities of 267.1 and 204.0 mg Trolox eq/100 g, respectively. These variations among the different samples may be related to differences in their freshness. It is reasonable to assume that the fish collected from the two department stores were fresher than that from the supermarket. Therefore, the result shown in Table 4 suggested that fresh cutlassfish has a lower radical-scavenging activity than the less fresh fish. However, the effects of the season of purchase or other factors cannot be excluded.

Since the radical-scavenging activity of cutlassfish was considerably higher than the activities of other fish species, a detailed study was made of the activity of this fish. A fresh fish was obtained from a department store and the activity of the edible part of the whole body was determined. The body was then divided into five regions: head, back, middle, abdomen and tail (Fig. 2), and a regional determination of the activity was performed on a fresh weight basis (Table 5). The activity of the tail region was the highest (253.2 mg Trolox eq/100 g) followed by the abdomen (217.2 mg Trolox eq/100 g), head (190.6 mg Trolox eq/100 g), back (184.7 mg Trolox eq/100 g) and middle region (158.7 mg Trolox eq/100 g). The activity of the skin region was very high as shown in Table 6. Therefore, the variation among the different parts seemed to arise from the percentage of the skin in the samples. Furthermore, samples closer to skin showed comparatively higher activities (Table 5). In addition, the muscle in the tail was very thin, and therefore the percentage of skin in the sample of the tail region was naturally higher than those in other parts of the fish.

The results shown in Table 5 indicated that the silver colored skin of this fish might contribute to its higher level of radical-scavenging activity. To test this hypothesis, the middle part of a fresh fish from a department store was separated into muscle and skin, and the activities of these two portions were compared with that of the whole fish sample (Table 6). A

**Table 5.** Regional radical scavenging activity of fresh cutlassfish.

Item	Radical scavenging activity (mg Trolox eq/100 g)		
	Water extract	Ethanol extract	Total
Head	149.8	40.8	190.6
Back	151.1	33.6	184.7
Middle	122.6	36.1	158.7
Abdomen	176.8	40.4	217.2
Tail	212.9	40.3	253.2

The data is presented on a fresh weight basis.

**Table 6.** Comparative radical scavenging activity of cutlassfish and halfbeak.

Item	Radical scavenging activity (mg Trolox eq/100 g)		
	Whole fish	Skin fish	Muscle
Cutlassfish	320.7	2365.7	91.0
Halfbeak	171.4	223.4	154.1

The data is presented on a fresh weight basis. Only the activity of water soluble extract has been used.

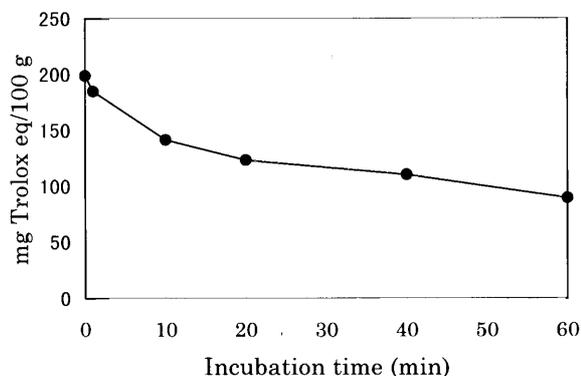
halfbeak which is very similar to the cutlassfish with respect to its tail color, was also analyzed and compared with the cutlassfish (Table 6). However, in the halfbeak, the variation among the different portions was not as prominent as was observed in the cutlassfish. The activity of cutlassfish skin, however, was extremely high compared with those of the other parts.

The skin of cutlassfish is covered with a silver color pigment known as guanine. This compound is used to shine artificial pearls. It has been reported that loss of this silver color pigment caused rapid deterioration in the freshness of the cutlassfish (Okabe, 1995). Therefore, the radical-scavenging activity of this fish was assumed to be related to the persistence of guanine on its skin.

*The radical-scavenging activity of guanine and its related compounds* Addition of artificial antioxidant for the preservation of raw foods is prohibited by the Food Sanitation Law in Japan. For this reason, the possibility of using additional antioxidant to preserve cutlassfish was not considered and the guanine was assumed to be a key compound for the extremely high radical-scavenging activity of this fish. A biochemical study revealed that the chief end product of guanine metabolism is uric acid. This compound has been reported to provide an antioxidant defense in humans against oxidants and radicals (Ames *et al.*, 1981). Therefore, the activity of guanine and its related compounds including uric acid were investigated (Table 7). Guanine, 5'-IMP, 5'-GMP, hypoxanthine and guanosine had negligible activities, however, while uric acid showed an activity as high

**Table 7.** Radical scavenging activity of guanine and related products.

Item	Radical scavenging activity (mg Trolox eq/100 g)
Guanine	0.09
5'-IMP	0.10
5'-GMP	0.12
Hypoxanthine	0.19
Guanosine	0.78
Uric acid	267.03
Trolox	250.29



**Fig. 3.** Effect of uricase on the radical scavenging activity of cutlassfish. Radical scavenging activity of fresh and intact cutlassfish was analyzed. Only the activity of water extract was used for comparison. The data is presented on a fresh weight basis.

as that of Trolox (a stable antioxidant). This result suggested that uric acid contributes to the radical-scavenging activity of cutlassfish.

*Effect of uricase on the activity of cutlassfish* In animals other than man and the anthropoid apes, uric acid is further degraded to allantoin (Mazur & Harrow, 1971). This compound did not show any radical-scavenging activity (data not presented). Uricase is responsible for the oxidative degradation of uric acid to allantoin. To determine the effect of uricase on the activity of cutlassfish, the water-soluble extract of a fresh fish was incubated with the enzyme for periods of 1, 10, 20, 40, and 60 min at room temperature and the activities were assayed. The activity decreased with increasing incubation time (Fig. 3). A 7% reduction was observed when the sample was incubated with the enzyme for 1 min, while 55% reduction was found in the case of 60-min incubation. This result indicated that the radical-scavenging activity of cutlassfish was reduced gradually with the degradation of uric acid to allantoin. However, the content of guanine or uric acid was not determined in the present investigation, therefore, the contribution of uric acid to the total activity cannot be quantified. Further studies are in progress.

Prevention of free radical-induced diseases through the consumption of foods having antioxidant activity is a great concern to diverse fields of research, particularly to food nutrition. Diets high in fruits and vegetables are associated with lower incidence of various of these diseases, especially coronary diseases and cancers. This study showed that fish also possessed considerable free radical-scavenging activities. Therefore, a balanced diet containing enough fish, vegetables and fruits could be the most effective in protecting the body from various oxidative stressors.

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