Short Paper

Distribution of Muscular Glutathione in Fish and Shellfish

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Glutathione (γ -L-glutamyl-L-cysteinylglycine, GSH) is known as an important multifunctional substance in human cells. GSH functions in the detoxification of various foreign materials such as heavy metals and carcinogens, and in the protection of cells against oxidative damage.⁽¹⁾ Although GSH is synthesized mainly in liver through the addition of glycine to the C-terminal of γ -L-glutamyl-L-cysteine, recent studies in humans suggested that orally taken GSH may be directly absorbed through buccal and intestinal mucosa and act as the functional component against oxidative stress.⁽²⁾ Therefore, GSH contents in our daily diets have become a matter of great concern. Saetre and Rabenstein⁽³⁾ reported that GSH contents range from 20 to 225 $\mu\text{mol/l}$ in various juices of fresh fruits and vegetables. Grazyna *et al.*⁽⁴⁾ analyzed 39 foods common in the United States and amounts of GSH are relatively high in meats (175–600 nmol/g wet wt) and moderate in fruits and vegetables (140–450 nmol/g wet wt). The GSH contents of various foods listed in the National Cancer Institute's Health Habits and History Questionnaire were systematically analyzed and relatively high amounts in freshly prepared meats were again reported.⁽⁵⁾ However, no integrated work is available on GSH concentrations in fresh fish and shellfish for human consumption. We describe here contents of GSH and its disulfide form in fish and shellfish widely consumed in Japan.

Dorsal portion of fresh fish and whole meat of shellfish purchased from a local supermarket in Kyoto city were immediately cut into small pieces. The 2 g of fish or shellfish sample was homogenized in ice-cold 10 ml 5% sulfosalicylic acid. Homogenates were centrifuged at 10,000 g for 5 min at 4°C and the supernatants obtained were stored at –70°C until HPLC analysis. For determination of GSH and its disulfide form, the supernatant was thawed at 3°C, diluted 10 times and injected into an HPLC system equipped with P1046 fluorescence detector, Yokogawa (em.350 nm, ex.420 nm). The GSHs were separated by TSKgel ODS80TM (150 mm $\times$ 4.6 mm, Tosoh) using 25 mM NaH_2PO_4 (pH 3.0) as a mobile phase at 50°C. The separated GSHs were simultaneously determined using the post-column technique using o-phthalaldehyde as a fluorescent reagent described by Fukunaga *et al.*⁽⁶⁾ All amounts were expressed on a wet weight basis.

The disulfide form of GSH (GSSG) is commonly pro-

duced by the action of glutathione peroxidase in fish and shellfish.^(7,8) However, when diets containing GSSG are taken, GSSG can be reduced into GSH in the gastrointestinal tract.⁽⁹⁾ GSSG as well as GSH was thus determined and total GSH was obtained as GSH + 2GSSG. Table 1 shows the amounts of GSH and its disulfide form (GSSG) and total GSH (GSH + 2GSSG) in 42 different species of fish and shellfish. In fish, the GSH contents ranged from 1.2 nmol/g to 255.6 nmol/g. In general, white-meat fish had lower contents of both forms of GSH than red-meat fish. Especially, angler, a sluggish fish, had extremely low contents of GSH and GSSG. Analysis of yellowtail, horse mackerel, and saury suggested that dark muscle had 2–3 times higher concentrations of total GSH than ordinary muscle. When compared with benthic fish, GSH levels in migratory or pelagic fish were relatively high, especially, yellowtail and salmon among migratory fishes had more than 180 nmol/g of GSH. Thus the fish living under the environmental and physiological conditions where active oxygens readily generate, seem to retain generally high contents of GSHs, although a definite statement can not be made because of the scarcity of collected data and the presence of some exceptional cases such as red sea bream, triggerfish, and Pacific cod. Further analysis is required to establish a precise relationship between GSHs' contents and the living conditions. Except for angler fish, the contents of GSSG were less than those of GSH.

Unexpectedly high amounts of GSHs occurred in shellfishes. Particularly, scallop showed considerably high level of total GSH. Although the significance of the high level is unknown, the enzymatic conversion of GSH into GSSG may be restricted due to a negligibly low activity of glutathione peroxidase in scallop.⁽⁷⁾ The GSHs' contents in fish and shellfish range from comparable to, much lower than those of animal meats (175–600 nmol/g wet wt).⁽⁴⁾ For Japanese who consume 37.9 g of fish and shellfish/year as a net supply,⁽¹⁰⁾ however, fish and shellfish may play an important role as a dietary source of GSHs. Since it is widely recognized that cellular constituents in fish and shellfish fluctuate highly as a result of seasonal and regional variations, or of differences between cultured and natural catches, GSHs amounts obtained here give tentative values of the components. Further studies are required to clarify pre- and post harvest factors including storage

Table 1. Distribution of muscular glutathione in fish and shellfish

Species		Glutathiones,* ¹ nmol/g			Remarks
		GSH	GSSG	Total GSH	
Vertebrates* ²					
Yellow tail	<i>Seriola quinqueradiata</i>	255.6	8.6	272.7	Dark muscle
Coho salmon	<i>Oncorhynchus kisutch</i>	181.2	15.1	211.4	Frozen
Yellow tail	<i>Seriola quinqueradiata</i>	138.4	3.4	145.3	
Red sea bream	<i>Pagrus major</i>	139.1	2.5	144.0	Cultured
Horse mackerel	<i>Trachurus japonicus</i>	120.6	2.4	125.5	Dark muscle
Albacore	<i>Thunnus alalunga</i>	113.2	5.2	123.6	
Bonito	<i>Katsuwonus pelamis</i>	100.3	9.9	120.1	
Yellowfin tuna	<i>Thunnus albacares</i>	110.4	2.8	115.9	
Triggerfish	<i>Stephanolepis cirrhifer</i>	88.3	10.9	110.0	
Horse mackerel	<i>Trachurus japonicus</i>	74.4	10.9	96.3	
Pacific cod	<i>Gadus macrocephalus</i>	73.3	10.7	94.7	Soft roe
Japanese stingfish	<i>Sebastes inermis</i>	70.4	3.1	76.6	
Saury	<i>Cololabis saira</i>	58.2	7.9	74.0	Dark muscle
Pacific cod	<i>Gadus macrocephalus</i>	56.5	3.0	62.6	
Striped jack	<i>Caranx delicatissimus</i>	45.9	5.6	57.1	
Japanese flounder	<i>Paralichthys olivaceus</i>	37.3	8.1	53.6	
Sardine	<i>Sardinops melanostictus</i>	27.6	10.2	48.0	
Threadfin bream	<i>Nemipterus virgatus</i>	40.2	3.5	47.2	
Japanese argentine	<i>Glossanodon semifasciata</i>	40.5	3.3	47.1	
Ocellate puffer	<i>Takifugu rubripes</i>	30.7	8.2	47.1	Cultured
Amberjack	<i>Seriola dumerili</i>	35.9	4.5	44.9	Cultured
Alaska pollack	<i>Theragra chalcogramma</i>	27.4	8.0	43.3	Salted roe
Japanese stingfish	<i>Sebastes inermis</i>	36.5	1.5	39.5	
Harvest fish	<i>Pampus argenteus</i>	25.1	4.0	33.0	
Saury	<i>Cololabis saira</i>	13.1	9.8	32.7	
Red flounder	<i>Hippoglossoides dubius</i>	26.6	1.9	30.3	
Sea bass	<i>Lateolabrax japonicus</i>	11.3	9.5	30.2	
Mackerel	<i>Scomber japonicus</i>	18.3	2.4	23.1	
Spanish mackerel	<i>Scomberomorus niphonius</i>	15.0	3.8	22.5	
Red horsehead	<i>Branchiostegus japonicus</i>	16.7	2.6	21.8	
Japanese butterflyfish	<i>Psenopsis anomala</i>	17.3	1.2	19.8	
Japanese conger	<i>Conger myriaster</i>	6.4	5.8	17.9	
Hairtail	<i>Trichiurus lepturus</i>	11.7	3.0	17.7	Frozen
Three-line grunt	<i>Parapristipoma trilineatum</i>	12.8	0.9	14.6	
Japanese whiting	<i>Sillago japonica</i>	3.9	2.8	9.5	
Angler fish	<i>Lophiomus setigerus</i>	2.1	2.6	7.2	
Conger pike	<i>Muraenesox cinereus</i>	2.1	0.9	3.8	Frozen
Angler fish	<i>Lophiomus setigerus</i>	1.2	0.7	2.6	Liver
Invertebrates					
Scallop	<i>Patinopecten yessoensis</i>	774.7	6.1	786.9	Frozen, Adductor muscle
Cockle	<i>Fulvia mutica</i>	107.4	56.3	219.9	Whole
Short-neck clam	<i>Ruditapes philippinarum</i>	159.8	24.2	208.3	Whole
Freshwater clam	<i>Corbiculina leana</i>	106.2	5.1	116.4	Whole
Cuttlefish	<i>Sepia esculenta</i>	81.6	6.8	95.1	Frozen
Hard clam	<i>Meretrix lusoria</i>	49.7	7.6	64.9	
Long-finned squid	<i>Loligo edulis</i>	0.9	10.5	21.9	
Pink shrimp	<i>Pandalus borealis</i>	12.8	2.9	18.5	Frozen
Purple sea urchin	<i>Anthocidaris crassispina</i>	1.4	4.6	10.5	Gonad

*¹ GSH:Glutathione, GSSG:Disulfide form of GSH, Total GSH:GSH+2GSSG, Average of three fish and shellfish.

*² Unless otherwise specified, dorsal ordinary muscle was analyzed.

stability and cooking loss that may cause the variation of GSHs' contents.

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