Recent Research Topics in the Laboratory of Marine Biochemistry

Tohoku Journal of Agricultural Research
Vol. 59 No. 3-4, March 2009
Printed in Japan

Recent Research Topics in the Laboratory of Marine Biochemistry

Minoru SATO, Toshiyasu YAMAGUCHI and Toshiki NAKANO

Laboratory of Marine Biochemistry, Graduate School of Agricultural Science, Tohoku University, Sendai 981-8555, Japan

(Received, March 2, 2009)

Summary

Foods are important for human by supply energy and sustain health. Sea foods are well known to contain unique constituents, such as n-3 polyunsaturated fatty acid EPA, DHA, which have various benefit for human health. Also, there are some trials to enhance fish health and quality to supply better cultured fish to consumers. On the other hand, sea foods deteriorate quickly than land animal products. It is needed, therefore, quick and simple methods to confirm the fish freshness and quality. In this short paper, recent topics in the laboratory of marine biochemistry will be stated.

Development of Quick and Simple Methods for Fish Inspection

Consumers are concerned about the characteristics of the food they eat on two main points : whether it is safe to eat and whether it has a good taste (Sato, 2004). The first point is an assurance that the food does not contain toxic substances or dangerous microorganisms, and the second is a demand for food with a good flavor. Fish flesh deteriorates much more quickly than meat of land animal. Freshness is an important determinant of the flavor and quality of fish and seafood products. There is a great demand on the quick and simple assay method for freshness and quality.

Histamine Checker

People get the food-poisoning called urticaria when take un-fresh fish. This is caused by histamine. Histamine is made by an amino acid, histidine, under microorganisms, histamine-forming bacteria (HFB) exist. Mackerel, bonito, and tuna contains high level of histidine, so that it is easy to generate histamine, it would cause histamine poisoning often. We developed new quick and simple analytical method for histamine determination in fish and seafood (Sato *et al.*,

2006). It consists of sample extraction, adsorption onto a paper disc, application of the paper disc onto electrophoresis paper, electrophoresis for only 5 min, drying and color developing by Pauly's reagent. Histamine can be satisfactorily detected and completely separated from histidine, carnosine, and other Pauly reagent positive compounds. This method does not need expensive instrumention and any tedious pretreatment to eliminate potential interference by other imidazole compounds such as histidine or carnosine. This method can be used to detect histamine in multiple fish and seafood samples simultaneously that contain as little as 15 ppm histamine.

We also developed quick HFB detection method by two-layer filtration method (Tao *et al.*, 2009). This method can be applied to food hygiene systems, including the hazard analysis and control critical point (HACCP) system in seafood processing lines.

Freshness Checker

Fish freshness is estimated by sensory, chemical, physical or microbiological methods. Among these methods, chemical methods are most objective and also K value, proposed by Saito *et al.* (1959), is now considered to be one of the most appropriate indicators of fish freshness. The main drawback of the method is that it requires an expensive instrumentation such as HPLC and a relatively long analysis time per sample. So, K value method is not widely used on fishery site. The new equipment "Freshness Checker" is based on the separation of nucleotide and nucleoside/base by paper electrophoresis and the calculation by the spot size and spot intensity of each compound. The former compound is contained mainly in fresh fish consisted ATP, ADP, AMP and IMP, and the latter compound is formed in deteriorated fish consisted inosine and hypoxanthine. K value can be easily calculated using the software "Spot Analyzer" (QS-Solution). It just requires about less 10 min for all measurement process (Sato, 2008a, b).

Lipids and Lipid Metabolism in Aquatic Organisms

The lipid composition and metabolism in fish and other aquatic organisms were investigated. Especially, polyunsaturated fatty acid (PUFA) metabolisms in gastrointestinal canal, liver or hepatopancreas, and blood plasma in those organisms were analyzed. The PUFAs were immediately incorporated into phospholipids or triacylglycerol after absorption, subsequently carried to liver. The phospholipids and triacylglycerol were decomposed in the liver. They release to the blood system after recomposition of phospholipids and triacylglycerol. The phospholipid concentration in the blood plasma lipoprotein in fish was higher than that of mammals. It is considered that phospholipids play important rules on the metabolism and transportation of lipids in fish. Bile salts and bile

alcohols were also important components on lipid absorption in fish (Yamaguchi *et al.*, 2000).

Characteristic lipid composition has already shown in the aquatic organisms. Alkyl ether lipids, that have ether bond in the molecule, were representative. We detected and identified alkenylacyl phospholipids in fish blood lipoprotein. It was investigated that the structural analysis and the biosynthetic mechanism of alkylacyl and alkenylacyl phospholipids. Although diacylglycerylether (DAGE) was uncommon lipid component in the terrestrial organisms, a large amount of DAGE was detected in some kind of aquatic organisms. And we also investigated on the effective utilization of DAGE (Yamaguchi *et al.*, 2002).

Antioxidative Defense against Stress in Fish

Carotenoids, which are usually yellow to red isoprenoid polyene fat-soluble pigments, are widely distributed in nature (Nakano *et al.*, 2003 ; Nakano, 2007, 2008). Most animals, including salmonid are unable to perform *de novo* synthesis of carotenoids. Accordingly, feed for cultured salmon should be supplemented with some kinds of carotenoids such as astaxanthin (ASX) to impart the desired muscle coloration. Recently, attention has been given to biological activities, other than the role in muscle pigmentation, of certain kinds of carotenoids in fish. In the course of studies on antioxidative defense in fish, it has been accepted that improvement in the cultured fish's potential of defense is important (Nakano *et al.*, 1993, 1995a, 2004). This is because cultured fish have many occasions to be exposed to stressors which cause oxidative stress in the body (Nakano and Takeuchi, 1997 ; Basu *et al.*, 2001 ; Nakano *et al.*, 2002). We have been researching on the effects of both synthetic ASX and ASX-rich red yeast *Phaffia rhodozyma* on oxidative states in trout (Nakano *et al.*, 1995b, 1999a, b ; Nakano, 2003 ; Nakano *et al.*, 2004 ; Nakano, 2007, 2008). For example, the liver, plasma and red blood cells from fish fed the ASX-supplemented diet were observed to have a significantly higher level of α-tocopherol and carotenoids than those from control fish fed a non-ASX diet. On the other hand, ASX significantly decreased lipid peroxides (LPO) levels in the tissues. Dietary red yeast and ASX were found to decrease the level of LPO in the serum of healthy trout (Nakano *et al.*, 1995b, 2004). Similar phenomena were also observed in the serum from oxidative-stressed trout (Nakano *et al.*, 1999a). Consequently, membrane-bound antioxidants such as α-tocopherol and ASX protect the membrane lipids and proteins in the tissues from oxidation. The GPT and GOT activities were lower in the serum of fish fed ASX than those of control fish (Nakano *et al.*, 1995b, 1999a). In addition, ASX could dramatically increase the viability in the reactive oxygen inductive-stressed cells (Nakano *et al.*, 2004). Hence, both ASX and red yeast are expected to improve liver function, increase the membrane's mechanical strength,

and further maintain membrane dynamics of the tissue against oxidative stress in fish.

References

Basu, N., Nakano, T., Grau, E.G., and Iwama, G.K., The effects of cortisol on heat shock protein 70 levels in two fish species. *Gen. Comp. Endocrinol.*, **124**, 97-105 (2001)

Nakano, T., Sato, M., and Takeuchi, M., Superoxide dismutase activity in the skin of fish. *J. Fish Biol.*, **43**, 492-496 (1993)

Nakano, T., Sato, M., and Takeuchi, M., Unique molecular properties of superoxide dismutase from teleost fish skin. *FEBS Lett.*, **360**, 197-201 (1995a)

Nakano, T., Tosa, M., and Takeuchi, M., Improvement of biochemical features in fish health by red yeast and synthetic astaxanthin. *J. Agric. Food Chem.*, **43**, 1570-1573 (1995b)

Nakano, T. and Takeuchi, M., The relationship between fish and reactive oxygen species. *Yoshoku*, **34(3)**, 112-115 (1997) (in Japanese)

Nakano, T., Kanmuri, T., Sato, M., and Takeuchi, M., Effect of red yeast (Phaffia rhodozyma) rich in astaxanthin on oxidative stress in fish. *Biochem. Biophys. Acta*, **1426**, 119-125 (1999a)

Nakano, T., Miura, Y., Wazawa, M., Sato, M., and Takeuchi, M., Red yeast Phaffia rhodozyma reduces susceptibility of liver homogenate to lipid peroxidation in rainbow trout. *Fish. Sci.*, **65**, 961-962 (1999b)

Nakano, T., Basu, N., Yamaguchi, T., Sato, M., Nakano, K., Hicks, J.M.T., and Iwama, G.K., Effect of cold shock and cortisol on heat shock protein levels in rainbow trout. *Fish. Sci.*, **68S**, 1037-1040 (2002)

Nakano, T., Yamaguchi, T., Sato, M., and Iwama, G.K., Biological effect of carotenoids in fish. *Proc. JSPS Intern. Seminar, Kaset Sart University, Bangkok, Thailand*, 26-41 (2003)

Nakano, T., Wazawa, M., Yamaguchi, T., Sato, M., and Iwama, G.K., Positive biological actions of astaxanthin in rainbow trout. *Marine Biotechnol.*, 6, S100-S105 (2004)

Nakano, T., Microorganisms, "*Dietary Supplements for the Health and Quality of Cultured Fish*" ed. by H. Nakagawa, M. Sato, and D.M. Gatlin III, CABI, Wallingford, UK, pp. 86-108 (2007)

Nakano, T., Effect of carotenoid on fish health. *Proc. 11th JMOA Marine Oil Technol. Congress*, Tokyo, Japan, 1-14 (2008) (in Japanese)

Sato, M., Fish inspection Freshness assessment, "*Encyclopedia of Meat Science*" ed. by W. Jensen, C. Devine, and M. Dikemann, Elsevier, Oxford, UK, pp. 474-482 (2004)

Sato, M., Tao, Z.H., Shiozaki, K., Nakano, T., Yamaguchi, T., Yokoyama, T., Kan-no, N., and Nagahisa, E., A simple and rapid method for the analysis of fish histamine by paper electrophoresis. *Fish. Sci.*, **72**, 889-892 (2006)

Saito, T., Arai, K., and Matsuyoshi, M., A new method for estimating the freshness of fish. *Bull. Jap. Soc. Sci. Fish.*, **24**, 749 750 (1959)

Sato, M., Freshness checker: quick and simple assay device for fish freshness. *Brain Techno News*, **128**, 24-29 (2008a) (in Japanese)

Sato, M., Development of a simple and quick measurement device for fish

freshness. *Aqua Culture Magazine*, **45**, 54–57 (2008b) (in Japanese)

Tao, Z.H., Sato, M., Abe, N., Yamaguchi, T., and Nakano, T., Simple and rapid determination of histamine-forming bacteria in differential agar medium. *Food Cont.* (2009) (in press).

Yamaguchi, T., Nakano, T., Takeuchi, M., and Sato, M., Plasmalogen of fish lipoprotein. *Fish. Sci.*, **68**, 1202–1205 (2002)

Yamagauchi, T., Miyamoto, K., Yagi, S., Horigane, A., Sato, M., and Takeuchi, M., Detection of plasmalogen from plasma low density lipoprotein and high density lipoprotein in carp, Cyprinus carpio and rainbow trout *Onchorrhynchus mykiss*. *Comp. Biochem. Physiol.*, **127A**, 339–346 (2000)