Utilization of Biological Responses of Fish and Shellfish for Improving Seafood Qualities

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Abstract
Quality control of seafood has been one major purpose of scientific researches on fish and shellfish. In this monograph, we deal with the utilization of biological responses in the preharvest and postharvest stages of aquatic animals, (1) responses of fish muscle energy metabolisms to environmental temperature changes in postmortem stages, (2) responses of fish and shellfish chromatophore to extrinsic stimuli, and (3) responses of fish to the oral administration of phytosterol related compounds. These approaches will not only confer the improvement of seafood qualities but the addition of extra value to seafood for human consumption. Because animals respond biologically to many factors, comprehensive understanding of biological responses of aquatic animals to environmental factors will lead to the further improvement of seafood qualities.

1. Introduction
Quality control of seafood has been one major purpose of scientific researches on fish and shellfish. Qualities including food function comprise safety for human consumption, nutritional values for humans, acceptability for humans, and benefit for human health. Micro-organismic risks are the most primary concern for safety of food (Chen 1995; Butt et al. 2004a, b; Walker and Winton 2010). Much attention is also paid to poisoning by sea food toxins (Saavedradelgado and Metcalfe 1993; Clark et al. 1999) and chemical contaminants (Taylor and Nordlee 1993; Neff 1997; Hofer 1998; Wegner and Baur 2005; Levenson and Axelrad 2006; Dorea 2008). Seafood allergy is now prevailing in the world due to increasing ingestion of seafood (O’Neil and Lehrer 1995; Lehrer et al. 2003; Stiller 2008; Lopata and Lehrer 2009). For attainment of food safety, HACCP (Tzouros and Arvanitoyannis 2000) and other management systems have been developed.

From the point of nutritional values, fish and shellfish are very important protein sources. Quality of the meat of fish and shellfish meats however, tend to deteriorate faster than the meat of terrestrial animals. Freshness is the most important factor for human acceptability of fish and shellfish. “Sashimi,” fish and shellfish raw meat, is popular in Japan and now widely accepted in the world. Sashimi needs extensively high freshness of course in micro-biological and also in biological aspects. Many storage methods including chilling and frozen storage, have therefore been developed for preserving fish and shellfish freshness. There are however some biological responses in early postmortem stages. Therefore, controls of such responses lead to the improvement of seafood quality. These approaches belong to postharvest controls, while we can utilize biological responses for quality improvement also in preharvest stages. For example, fish accumulate polyunsaturated fatty acids in their bodies, which raises the possibility that fish and shellfish might positively accumulate some physiologically important substances via culture diets, conferring higher qualities on the seafood products.

In this monograph, we deal with the improvement of seafood quality by utilizing the biological responses in pre- and post-harvest stages of fish and shellfish.
2. Rigor mortis progress

Rigor mortis is one of the most obvious phenomena in fish muscle postmortem. Because fish muscle in rigor state is stiff but fragile, strong external forces in handling easily destroy the muscle structure and, consequently, spoil meat texture. Such fragility is also attributed to weak texture in masticating in mouth. Prevention of rigor mortis progress is important for the preservation of meat texture in highly fresh fish muscle, such as "Sashimi.

ATP is an important energy compound for sustaining biological activities. When ATP production is stopped in postmortem stage, ATP is broken down by ATPases including membrane pumps and myofibrillar ATPase and is consequently exhausted up. The ATP exhaustion in muscle cell precludes the dissociation between myosin and actin filaments, a rigor state. In many cases, ATP degradation accelerates with increasing temperature as general chemical reaction. In skeletal muscle postmortem, however, ATP is degraded faster at ice-cold temperature than at 5–10 degrees C, resulting in an accelerated rigor mortis progress (Watabe et al. 1989a). Skeletal muscle contraction is controlled by cytosolic Ca$^{2+}$ via the troponin-tropomyosin regulatory system. Cytosolic Ca$^{2+}$ concentration is modulated by intracellular Ca$^{2+}$ store, sarcoplasmic reticulum (SR) (Ushio et al. 1989; Ushio and Watabe 1993). SR uptakes Ca$^{2+}$ by Ca$^{2+}$ pump, sarco/endoplasmic reticulum Ca$^{2+}$-ATPase (SERCA), to reduce cytosolic Ca$^{2+}$ concentration, resulting in muscle relaxation. In a contracted state, SR releases Ca$^{2+}$ through Ca$^{2+}$ channel of SR, ryanodine receptor (RR) to increase cytosolic Ca$^{2+}$ concentration. We demonstrated that SERCA activity is reduced and RR is frozen at an opened state at low temperature such as ice-cold temperature and that cytosolic Ca$^{2+}$ concentration drastically increases (Ushio et al. 1991). The ice-cold temperature in turn accelerates ATP breakdown by myofibrillar ATPase, even though myofibrillar ATPase activity is also reduced at that temperature (Iwamoto et al. 1988; Watabe et al. 1989b, 1990a). Cytosolic ATP is consequently exhausted and rigor mortis progresses at this temperature, so-called ice-cold contraction (Watabe et al. 1989b) (Fig. 1). Thus, precise temperature control is also important for controlling fish meat texture in highly fresh muscle sustaining biological activities.

In eurythermal temperate fish such as carp Cyprinus carpio and goldfish Carassius auratus, temperature acclimation of physiological changes come about in order to compensate for the effects of temperature variation on muscle contractility and energy metabolisms (Hazel and Prosser 1974). The temperature acclimation leads to temperature-selective gene expressions of myosin heavy chains (Watabe et al. 1995; Imai et al. 1997; Cole and Johnston 2001) and mitochondrial ATPase subunits (Itoi et al. 2003). Fish acclimated to lower temperature show higher myofibrillar ATPase activity (Watabe et al. 1990a; Hwang et al. 1991), but higher intracellular ATP concentration due to enhanced mitochondrial FoF1-ATPase (Itoi et al. 2003) and SERCA activities (Ushio and Watabe 1993) together with slower rigor mortis progress (Fig. 2).

Because SERCA is a membrane intrinsic protein, the activity is altered by membrane environment, fluidity and fatty acyl chains of phospholipids. We then performed the manipulation of membrane fatty acid composition of SR through oral administration of fatty acids such as n-3 and n-6 polyunsaturated fatty acids in order to change Ca$^{2+}$ uptake activity of rainbow trout SR. SR of rainbow trout administered with the diet containing corn oil, rich in n-6 fatty acids, such as li-
noleic acid, showed n-6 fatty acid rich compositions (Ushio et al. 1997). The fish ingesting the diet containing fish oil, rich in n-3 fatty acids, such as docosahexaenoic acids, exhibited n-3 rich fatty acid profiles in the SR. Ca^{2+}-ATPase activity of n-6 rich SR was higher than that of n-3 rich SR (Fig. 3). Control of dietary fatty acids might thus allow us to modulate the SR Ca^{2+}-ATPase activity and therefore post-mortem changes in fish muscles.

A Japanese traditional cooking way, “Arai” in Japanese meaning washing of raw fish meat, is also one of controlling techniques of fish meat quality, texture. Short-term heat treatment around 45 degree C induced a partial inactivation of tropomyosin/troponin regulatory system in carp skeletal muscle, partial contracture, ATP exhaustion, and rigor progress (Watabe et al. 1990b), conferring the special texture of Arai (Fig. 4). Inactivations of SERCA and RR of carp muscle by the short-term heat treatment also contributed to the increase in cytosolic Ca^{2+} concentration, resulting in the contracture and rigor progress (Ushio and Watabe 1994).

3. Skin color control

Skin color of fish and squid is also one of factors affecting consumer acceptability. Skin colors of fish and squid are rapidly altered in the response to environmental stimuli, such as background color, light intensity, or changing social context.

Fish skin color is controlled by chromatophores; melanophores (black or brown), xanthophores (ocher or yellow), erythrophores (red), leucophores (whitish), cyanophores (blue), and iridophores (metallic or iridescent) (Fujii 2000). The former 5 species of chromatophores contain pigment granules and move the chromatosomes for color expression. Iridophores have multilayers of thin guanine plates and change the distance between plates for modulation of light-reflecting characteristics.

Wild red sea bream has vivid red skin color, which is indeed valued in consumer markets. Many investigators have improved the skin color by dietary control, such as feeding astaxanthin-enriched diet (Fujita et al. 1983; Shahidi et al. 1998) and the resulting skin color of the fish is very vivid in the living state. Immediately after killing the fish for consumption, however, the color quickly becomes dark, reducing the commercial value of the fish. The frequent melanophore occurrence in cultured fish skin and the melanophore activity immediately after death are responsible for the darkened skin color. The prevention of melanophore occurrence in the fish skin is partially protected by sunshading (Adachi et al. 2005), while the activity of chromatophores including melanophore is modulated via hormonally, neurophysiologically, and physical stimuli such as light (Fujii and Oshima 1986; Fujii 2000). Chromatophores of fish keep their biological activities and respond to extrinsic stimuli immediately after death like other tissues. We then demonstrated that high potassium ion concentration elicited the depolarization of neuron and chromatophore plasma membrane, inducing melanosome aggregation in melanophores (Lin et al. 1998c). Ice-cold temperature treatment induced melanosome aggregation in melanophores of red sea bream skin, but erythrophore dispersion in erythrophores, resulting in expression of vivid red color (Lin et al. 1998a) (Fig. 5). On the other
hand, in splendid alfonsino *Beryx splendens*, which also express vivid red skin color, high potassium ion concentration leaded the skin color to the erythosome aggregation and consequently to the faded color (Lin et al. 1998b). Light also induces chromatosome movement in chromatophore (Fujii 2000). The primary light responses are observed in the embryonic or larval chromatophores not yet controlled by hormonal or nervous systems. Chromatophores denervated also respond to light directly. In red sea bream chromatophores, isolated melanophore responded to light and melanosomes were dispersed. On the other hand, erythrosomes in isolated erythrophore were aggregated in response to light. In Nile tilapia *Oreochromis niloticus*, either innervated or denervated erythrophores responded directly to light and erythrosomes dispersed around 470–530 nm, while erythrosomes aggregated in other ranges (Oshima and Yokozeki 1999). These results suggest that Perciformes fish chromatophores have developed several intracellular signaling systems for response to light.

Squid skin color with black or dark brown patches are sometimes observed, which look like a giant panda (Fig. 6) and markedly reduces commercial values. Squid skin color is modulated by thousands of chromatophore organs with the retraction and the expansion of their pigment sacs under direct neuromuscular control (Cloney and Florey 1968; Florey 1969). A chromatophore organ contains various cells, such as a central cell with a pigment sac, a set of 6 to 20 radially arrayed muscles, and their sheath cells (Cloney and Florey 1968). Contraction and relaxation of the radially arrayed muscles induce the expansion and the retraction of pigment sacs, respectively. An energy loss in the chromatophore organs causes relaxation of the radially arrayed muscles and retraction of the chromatophore sacs, resulting that squid skin color gradually becomes white after death. The retraction of pigment sacs in partial skin area leads to the above giant-panda-like appearance.

We then investigated effects of chilling and hypoxia treatments on skin color of Japanese common squid *Todarodes pacificus* and spear squid *Loligo bleekeri* (Okada et al. 2004). Skin color of the squids gradually faded away during ice-cold storage. Chilling treatment of squid skin preparation caused muscle relaxation and the resulting retraction of pigment sacs and hypoxic treatment also induced pigment sac retraction. Covering treatments of squid skin with several plastic films of different permeabilities against oxygen suggested that interruption of oxygen supply by low oxygen per-
meable plastic films such as polyvinyliden chloride film caused squid skin whitening (Fig. 7). From these results, skin color can be maintained well after death by keeping the bodies well-oxygenated and avoiding direct contact with ice.

4. Accumulation of phytosterol related compounds in fish muscle

The above descriptions belong to the postharvest utilization of biological responses. We can also utilize biological responses in preharvest stages for quality improvement. Accumulation of physiologically functioning compounds in fish and shellfish muscles will lead to quality improvements and high commercial values as in the case of polyunsaturated fatty acids.

It is generally accepted that the amount and balance of lipid and carbohydrate, not only protein, affect feed utilization and protein retention of culture fish (Hillestad et al. 1998; Caballero et al. 1999; Hemre et al. 2002). Carnivorous teleost fish is also traditionally considered as glucose intolerant (Moon 2001), just like our type 2 diabetes. Control of lipid and carbohydrate metabolisms in teleost will allow us to manipulate feed utilization and protein retention of culture fish more efficiently.

Phytosteryl hydroxycinnamates (PSHCEs), observed in plants, play diverse and critical roles in plant architecture and defense. They are present as structural components of cell walls, contribute to interact among plants, and are precursors in the synthesis of flavonoids. PSHCEs have some physiological functions. One of the major bioactive PSHCEs in rice bran oil, gamma oryzanol (ORZ), is a mixture of plant sterol and ferulic acid esters (Xu and Godber 1999; Islam et al. 2011).

We have recently revealed that ORZ suppresses NF-kappaB activation, consequently inhibiting inflammatory responses of macrophages (Nagasaka et al. 2007) and that PSHCEs, in special ORZ, enhanced secretion of adiponectin, anti-type 2 diabetes cytokine (Kadowaki et al. 2006), in mice and 3T3-L1 adipocytes (Ohara et al. 2009). ORZ possibly suppresses factors for type 2 diabetes development induced by dietary fat (Nagasaka et al. 2011b) and stress (Ohara et al. 2011). We have then investigated the influence of oral administration of ORZ on rainbow trout energy production from lipid and carbohydrate. Body weight gain was expedited by ORZ administration (Fig. 8) (Nagasaka et al. 2011a).
et al. 2011a), probably because ORZ improved energy production from lipid and carbohydrate. It was also revealed that ORZ administration induced its accumulation in skeletal muscle of rainbow trout, yellowtail and red sea bream, providing us with accumulation of dietary ORZ by fish, “biological extraction” (Figs. 9–11) (Nagasaka et al. 2011a).

The absorption and draining pathways for ORZ in teleosts are still not clear. In mammals, the intestine has a barrier that prevents the absorption of plant sterols and their derivatives with structures similar to cholesterol (Yang et al. 2004). They are transported through Niemann–Pick C1-Like1 (NPC1L1) in intestinal mucosa cell (Davis et al. 2004). Acyl-CoA:cholesterol O-acyltransferase 2 (ACAT2) presents a strong priority for cholesterol rather than plant sterols (Temel et al. 2003), while dietary plant sterols are accumulated in human patients with mutations in either of the transporters ATP-binding cassette (ABC) transporters g5 and g8 (Klett and Patel 2004). In mammalian liver, the ABCg5/8 transporter expressed and mediates excretion of plant sterols into bile (Graf et al. 2003; Plösch et al. 2004) and plant sterols are hardly observed in skeletal muscles. However, the classical studies demonstrated that plant sterols were observed in fish skeletal muscle (Kritchevsky et al. 1967; Morris et al. 1982). Though ORZ transporters are still unclear in mammals and in fish, it is thought that difference of barrier and transport systems might cause the ORZ accumulation in fish muscle. These “biological extractions” might be useful for our ORZ intake and consequently improvement of commercial value of the culture fish.

5. Conclusion

This monograph has described the utilization of biological responses in the preharvest and postharvest stages of aquatic animals for the improvement of seafood quality, including the addition of another value for human consumption. Because animals respond biologically to other factors than the factors described...
here, comprehensive understanding of biological responses of aquatic animals to environmental factors will lead to further improvement of seafood quality.

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