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Freshness and Quality of Fish and Shellfish (Supplementary Edition)

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Abstract

To evaluate the freshness of fish and shellfish, the decomposed compounds from ATP in their muscles were analyzed quantitatively with a lapse of storage time. The K value of muscle was then demonstrated as B/A×100, where B is the amount of inosine plus hypoxanthine and A is the sum amount of ATP-related compounds. The onset of rigor mortis and following shortening of the muscles from aquatic animals are known to occur very earlier than those from terrestrial animals, and the content of ATP is closely related to the changing in development of rigor mortis of the muscles. Consequently, the method for evaluating the freshness of these muscles by using the K values has become popular especially in Japan, because the fresh fillet of these animals in rigor state were suitable to be raw materials of "Sashimi" and "Sushi".

In addition to the K value, other appropriate evaluating indices like as organoleptical, physical, biochemical, and microbial tests depending on the type of processed muscle food product have to measure. The synthetic evaluation by using several indices used to give us more reasonable evaluation of the quality of product.

Keywords: Fish and Seashell, Freshness, Quality, Kvalue

Preface

In order to evaluate the freshness of fish and shellfish, the ATP decomposition products in the muscles have been widely quantitated durnig storage. The K value was expressed as a ratio (%) of the total quantity of (inosine + hypoxanthine) against the total quantity of ATP-related compounds. It is known that rigor mortis of marine animal muscles and the subsequent muscle softening begin markedly faster when compared with land animals. The ATP content in the muscles is closely related to changes in the rigor mortis of muscles. A method for evaluating the freshness of these muscles using the K value has consequently become popular in Japan, as it is preferred to use seafood samples in solid condition for use in "Sashimi" and/or "Sushi". For some processed products, it is necessary to use more appropriate evaluation indices, such as organoleptic, physical, biochemical and microbiogical testing, because quality evaluation of products using more indices can provide clearer results.

In this connection, this paper is a revised edition of the present: Rigor mortis of fish and shellfish and evaluation of freshness of their muscles as K value, in Journal of The School of Marine Science and Technology, Tokai University, 4, 31-46, by Koseki, S. *et al*. (2006).

Contents

After death, biochemical changes occur in the muscles of animals, and these changes differ from those in living animals. Muscles are organs that use chemical energy (as shown in Fig. 1) in the form of ATP (adenosine 5'-triphosphate), and the muscles regenerate ATP as it is consumed. After death, however, the muscles lose the

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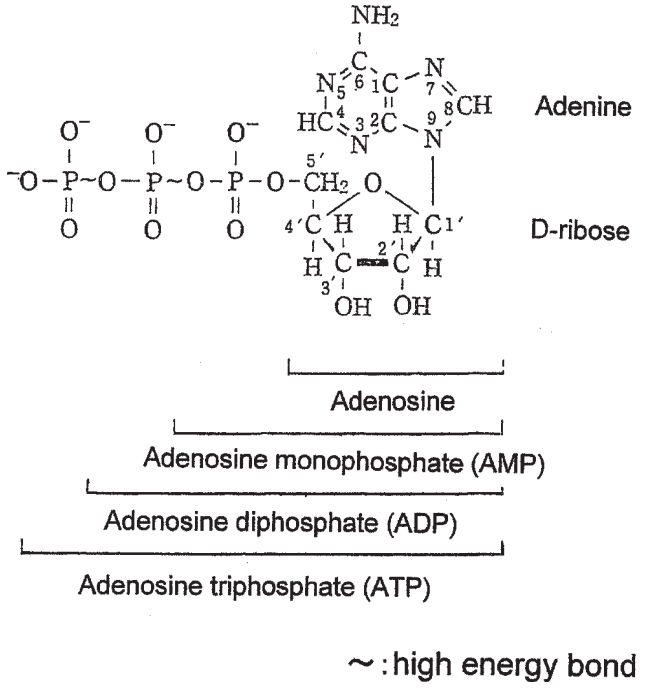

Fig. 1 ATP with bioenergy

ability to regenerate ATP. This greatly affects the shelf life of fresh food and processed food materials. Therefore, it is important to understand the various biochemical changes occurring in fish food for quality evaluation and quality control.

1. Post-mortem changes of fish and shellfish.

Muscles of living animals are flexible and clear, but become hardened and unclear after death. This phenomenon is known as rigor mortis. The length of time after death before rigor mortis is generally 24 hours for cattle, 12 hours for swine and 2 hours for chickens. The duration of rigor mortis at 5°C is eight to ten days for cattle, four to six days for swine and 12 to 24 hours for chickens. In contrast, fish meat enters rigor mortis between a few minutes and ten hours after death, with a duration of 5 to 22 hours (as shown in Fig. 2). However, the reasons remain unclear.

The time of rigor mortis onset and its duration differ because rigor mortis depends on the speed of the various biochemical reactions occurring in the muscles, and is affected by the animal species, nutrition and storage conditions. The main biochemical reaction involved in rigor mortis is the reduction of phosphagen and glycogen. The phosphagen in fish meat is creatine phosphate, while the phosphagen in invertebrates is arginine phosphate. ATP is consumed during the onset

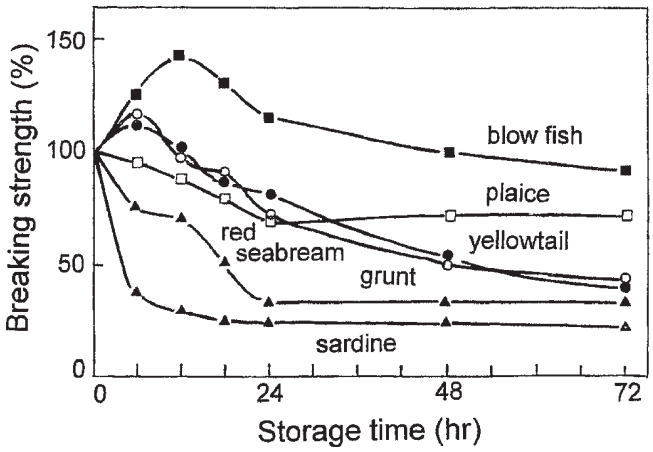

Fig. 2 Change in hardness of various fish muscles during iced-storage (K Hatae, 1995)

of rigor mortis, and the generated ADP is converted back to ATP by the phosphagen and phosphotransferase. Myokinase-mediated pathways regenerating ATP from ADP are also in operation. On the other hand, glycogen produces ATP by metabolism to lactic acid via glucose under anaerobic conditions. Glycogen further produces ATP under aerobic conditions via the action of mitochondria, which are numerous in muscle. Due to these supplemental regeneration mechanisms, the ATP content of muscle is maintained for some time after death. However, the muscles stiffen as phosphagen and glycogen, and eventually ATP, disappear. The timing of ATP loss closely corresponds to the onset of muscle rigor.

Muscles in living animals have a pH between 7.2 and 7.4, which decreases with glycolysis. Generation and accumulation of lactic acid during glycolysis, and the generation of H^+ during ATP production also play a role. The decrease in pH after death greatly depends on the glycogen content. Benthonic white fish meat has a low glycogen content (less than 0.4%) and the pH is between 6.0 and 6.4, while anadoromous red fish meat has a high glycogen content (around 1.0%) and the pH decreases to between 5.2 and 5.6. Shellfish muscles exhibit a similar pattern. If the pH in the muscles decreases, Ca ions leak from the endoplasmic reticula and mitochondria, and denaturation of myofibrillary proteins also occurs, thus affecting muscle rigidity.

The structure of the muscles in rigor mortis is largely the same as that of live muscles, with shorter sarcomeres owing to slips between the thin and thick filaments, except that the contraction of the live muscle is reversible while that during rigor mortis is irreversible. During muscle rigor, Ca ion leakage occurs because

ATP, which is necessary for Ca^{2+} accumulation in the endoplasmic reticula, is lost. In other words, it may be possible to estimate the degree of rigor by measuring Ca ion concentration, as Ca ion concentrations rise to around 10^{-4} M during muscle rigor, while those in live muscle are around 10^{-6} M.

As mentioned above, muscle rigor after death is strongly related to a series of biochemical changes. It is generally dependent on temperature. This suggests that ATP loss, and the speed and degree of rigor vary with temperature. However, as discussed below, under abnormal conditions that damage the endoplasmic reticula and mitochondrial membrane, leading to Ca ion leakage, special attention is needed, as ATP loss and the speed of rigor vary (see Fig. 3).

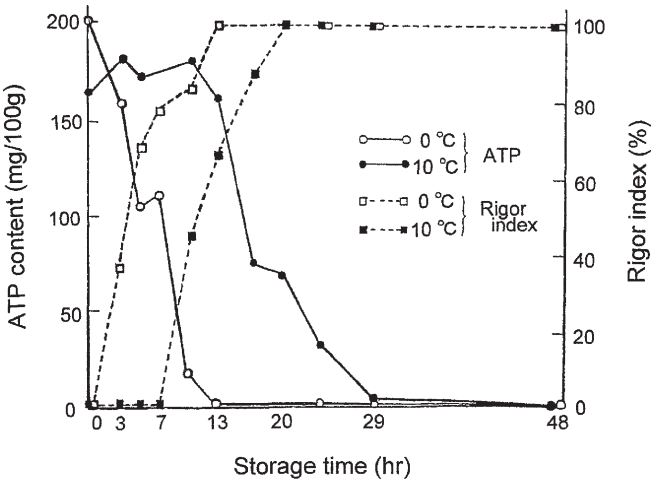

Fig. 3 Change in ATP content and rigor index of red seabream during chilled-storage (H Yamanaka, 2002)

Muscles become most rigid when ATP is lost and rigor is completed, before softening with time. This is known as rigor off. In the case of livestock, muscle requires a period of aging before being supplied as food, as the rigor is reversed extremely slowly. Thus, studies related to the process between rigor and softening have been emphasized. In contrast, in fish and shellfish, rigor off concurrently occurs with biochemical reactions and softening occurs easily. Accordingly, delaying the onset of rigor is emphasized. As a softening factor, substantial attention has focused on the action of endogenous proteases in the muscles. This process is also referred to as autolysis. However, several points remain unresolved, such as the ratio of generated amino acid and peptide quantities and the process of digestion and decomposition. In aging, fragmentation of myofibrils occurs due to weakening of the Z line. Quantitative analysis is also needed for relation between these changes and changes in rigidity and softening. There are various theories as to the cause of Z line weakening, including the muscle tension occurring at the time of rigor, structural collapse caused by interactions between the Z line and Ca ions, as well as digestion and the decomposition by endogenous protease.

It has been verified that the bond between actin and myosin filaments formed at the time of the rigor is weakened, as the dissociation of myosin filaments drastically increases in the presence of Ca ions and a protein affecting the dissociation is present that combines with actin filaments. Another theory also suggests the involvement of connectin (connective protein between muscular cells) and the decomposition of collagen (connective protein of muscular cell periphery tissue), which binds muscular tissues, in muscle softening, but the details of this involvement mechanism are unclear.

The onset and duration of muscle rigor after death are affected by various factors. There are several phenomena affect muscle properties after death. One of these is cold shortening, which significantly shortens the muscles by freezing at 10°C or below. Cold shortening is caused by Ca ion leakage from the endoplasmic reticula and mitochondria, while the pH and ATP content remain similar to those in the muscles of living animals. Another phenomenon is thaw rigor, in which the muscles of animals are significantly shortened after quickly being frozen and thawed. ATP and glycogen are rapidly decomposed on thawing to generate lactic acid and inosinic acid, and this rapidly lowers the pH in the muscles. This event is thought to promote Ca ion leakage from the muscle endoplasmic reticula and mitochondria, which causes rigor. If such animal muscles are stored for two weeks at −15°C and ATP is lost under the frozen conditions, no rigor occurs. Such variations in the conditions after death affect the process of the muscle rigor.

Fish and shellfish exhibiting rigor mortis and rigor off were concomitantly exposed to the infection of microbes, just after being caught. Initially, the infection is slow, but gradually becomes more rapid, causing deterioration of color and gloss, abnormal odor and taste, and sometimes generating harmful substances. This process is referred to as putrescence. Generally, the muscles of fish and shellfish are characterized by earlier putrescence because they comprise softer tissues with a high water content, and are more easily infected by

microbes than livestock. Accordingly, special attention is needed to control microbes in the case of fish and shellfish muscles.

Along with the increase in microbes, muscle components change in quality or are decomposed, and the patterns of these changes are complex. An example of the changes during the ice-storage of instantly killed plaice muscles is shown in Fig. 4. In the case of white muscle fish, pH changes are relatively small. Volatile base nitrogen levels increase and peak within a few days, while free amino acid levels increase after a few days. However, increases in K value under the same conditions begin after 12 hours and peak after 28 hours. Thus, the component changes during putrescence proceed at an extremely slow rate.

2. Freshness evaluation of fish and shellfish.

The freshness of fish and shellfish must be judged accurately and objectively, as freshness is needed to determine utility. Various methods of judging freshness have been considered, and each has its advantages and disadvantages. At present, there is no quick and useful method that can be applied to all types of fish and shellfish to judge freshness. It is important to understand the meaning of the indices used in these methods before selecting the most appropriate method. Here, the major methods proposed to date, a recent method using K value, and the classical method, are described.

Organoleptic methods

Examining the properties on an object using the human senses is referred to as organoleptic testing. Depending on the item, human senses can be highly accurate. This method is suited for overall judgment, and is based on appearance, and abnormal taste and odor. However, careful selection of the panelists and clear evaluation standards are required, in order to ensure objectivity in quantifying the results.

Chemical methods

This method measures substances that are absent in live fish and shellfish muscles, but are generated during deterioration. The criteria measured include volatile base nitrogen (NH_3, trimethyl amine, dimethylamine and the like), pH (dependent on lactic acid and volatile base nitrogen formations), decomposed products from ATP, organic acids, nonprotein nitrogen, histamine, and

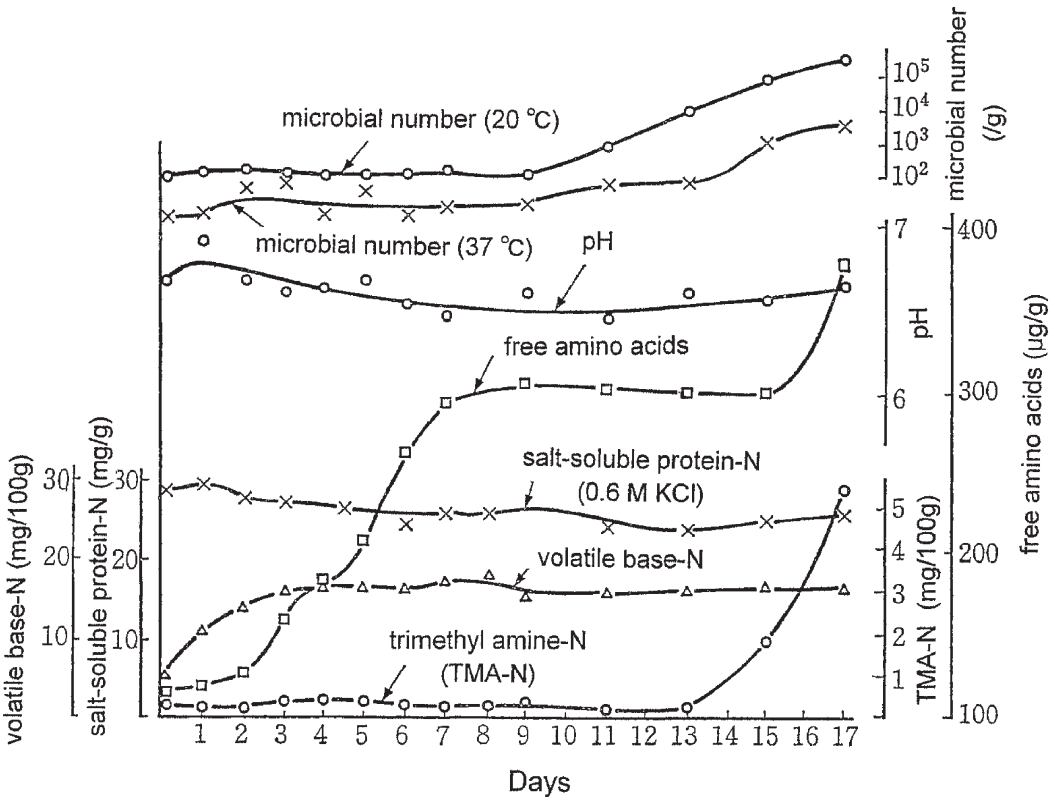

Fig. 4 Change in microbial number, pH, free amino acids, salt-soluble protein-N, volatile base-N, and trimethyl amine-N (TMA-N) during iced-storage (Uchiyama *et al*, 1966)

indole, etc. Empirical standards for fish and shellfish freshness suggest that volatile base nitrogen values of 5 to 10 mg/100 g indicate extreme freshness, 15 to 25 mg/100 g indicate ordinary freshness, 30 to 40 mg/100 g indicate initial putrescence, and 50 mg or over/100 g indicate putrescence. In comparison with ATP decomposition (increase in K value), however, these changes are considered to be quite slow (Fig. 5).

Structural proteins and lipids are constituents of fish and shellfish muscles, and they denature as muscle deterioration. Generally, when protein denatures, solubility and functionality change. In lipids, constituent unsaturated fatty acids are easily oxidized. Accordingly, if a product contains lipids, its quality will be affected. Changes in protein and lipid content must therefore be accurately evaluated. The main material in fish paste products, such as "Kamaboko", is myofibrillar protein, while that in dried fish are myofibrillar protein and lipid. For fish, protein in frozen Surimi, salt solubility, ATPase activity of myosin and gelation ability are good quality indices. For dried fish, water retentivity, water activity, and degree of lipid oxidation are good quality indices. Changes in these indices are not related to the decomposition of ATP (K value) in fresh fish meat, and proceed separately at relatively slow speed in the complex reaction mechanism.

Physical methods

This method is based on the hardness of the fish body and changes in electric resistance of it. A method of using the hardness of a fish body (rigor index) quantifies the phenomenon of muscle stiffening and subsequent softening. This method is valued because of its compatibility with the consumption of raw fish and shellfish meat. However, it is difficult to ensure accuracy as hardness can exhibit marked variations depending on species, the live fish physiology, nutrition, and the circumstances surrounding death. A method based on the induction ratio of fish meat, as measure with a Torrey meter, cannot provide an accurate evaluation, as there are significant differences among fish species. For such indices, fundamental consideration is required.

Microbiological methods

Putrescence of fish and shellfish is caused by microbial contamination and occurs irrespective of changes in other indices, such as chemical indices that measure changes in the original components of fish and shellfish muscles. However, the freshness of fish and shellfish is reflected to some extent by increases in the number of living microbes, as this number is closely related to the process of putrescence. When fish meat reaches initial putrescence, a strong putrid odor is detected, and the number of living microbes increases to 10^7 to $10^8/cm^2$. However, such data must be considered together with changes in other indices, as the number of living microbes varies with storage conditions. Consumer awareness regarding food safety has also increased in

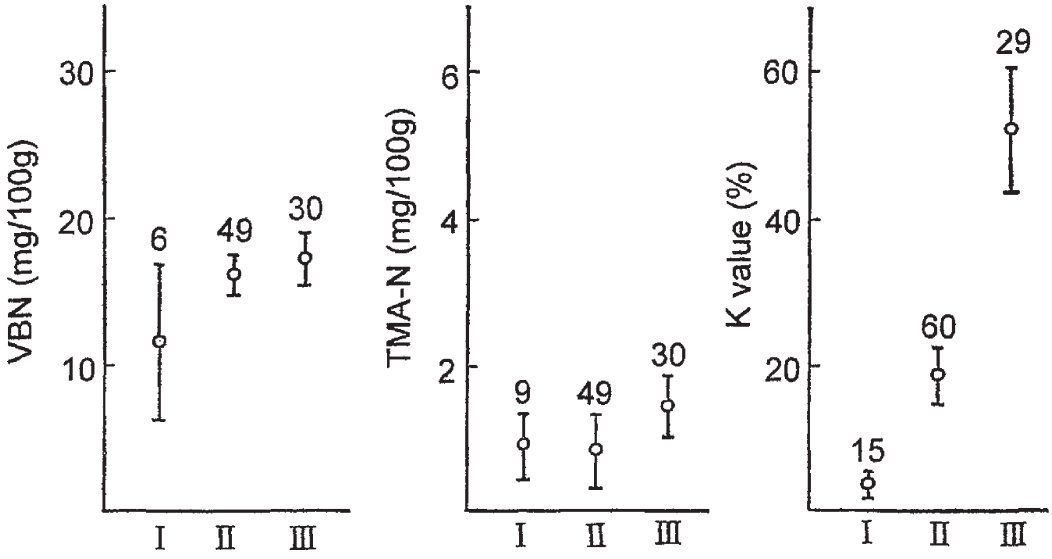

Fig. 5 Relation among volatile base-N, trimethyl amine-N (TMN-N), and K value of fish muscle of different species.
I: Fresh muscle. II: Raw muscle for sushi (excellent).
III: Raw muscle for sushi (good). Figures: Number of test.
(Uchiyama et al, 1970)

recent years, and thus the introduction of HACCP (Hazard Analysis Critical Control) is a barometer for interest levels in food safety in the aquatic food industry. Because consumption of fish and shellfish is increasing in Europe and the United States, where people tend to be cautious with regard to food safety, it is necessary to ensure comprehensive quality control, including K value, for seafood exported from Japan.

3. Freshness judgment by K value.

Muscles are motor organs that use ATP as an energy source. A large amount of ATP is present in the muscles of fish and shellfish (Table 1). After death, ATP is lost as the mechanisms of ATP regeneration cease to function, and thus changes in ATP are widely used as a freshness index. According to analysis of ATP decomposition in fish and shellfish muscles, ATP decomposes as follows: in fish muscles, ATP - ADP - AMP - IMP - inosine - hypoxantine; in the muscles of cephalopods and shellfish, ATP - ADP - AMP - adenosine - inosine - hypoxantine. In crustaceans, decomposition follows both paths. It is also known that shellfish muscles retain ATP for longer periods when stored at room temperature, that ATP regeneration (glycolysis reaction) continues to operate for some duration, and that in the muscles of cephalopods and crustaceans, the generation and accumulation of hypoxantine rapidly occurs. Therefore, the total value of (inosine + hypoxantine) as a percentage of all components analyzed is measured as an index (K value) for freshness of fish and shellfish (Fig. 6). In fish muscles, there are several enzymes that decompose ATP to hypoxanthine (Fig. 7). We have found that fish muscles contain AMP-deaminase but no adenosine deaminase, while shellfish and cephalopod muscles contain the latter but not the former. Crustacean muscles contain both enzymes. However, it is not yet clear why the enzyme group differs with animal species. Even if antibiotics are added to the muscles during storage, the ATP decomposition patterns remain unchanged. The above enzyme groups are inherent to the muscles. Because 5'-nucleotidase activity is inhibited at low temperatures, muscles in storage are characterized by the generation and accumulation of large amounts of IMP (inosinic acid) or adenylic acid. These components are known as taste components.

In addition to the K value, revised values, such as K' value and Ki value, are used as indices. For K' value, the numerator is amended to the total value of (inosinic acid + inosine + hypoxanthine), while for Ki value, the denominator is amended to the total value of (inosinic acid + inosine + hypoxanthine). In some cases, it is more convenient to use these values as indices, for

Table 1 The amount of acid soluble nucleotides in fresh muscle of some marine invertebrates (μmole /g muscle wet wt.)

	ATP	ADP	AMP	IMP	HxR	Hx	Total	Muscle
squid, *Ommastrephes sloani pacificus*	7.48	1.53	0.55	0	0	0.01	9.57	Mantle
	8.28	1.52	0.50	0	0	0.01	10.31	
squid, *Doryteuthis bleekeri*	3.12	2.42	2.19	0	0	0.19	7.92	Mantle
octopus, *Polypus dofleini*	4.00	1.02	0.20	0	0	0	5.22	Arm
scallop, *Pecten yessoensis*	6.02	0.87	0.59	0	0	0	7.48	Adductor
	6.40	1.20	0.60	0	0	0	8.20	
akazara, *Chlamys nipponensis akazara*	6.53	1.10	0.43	0	0	0	8.06	Adductor
	6.07	1.24	0.67	0	0	0	7.98	
surf clam, *Spisula sachalinensis*	3.98	0.50	0.33	0	0	0	4.81	Foot
	4.76	0.50	0.52	0	0	0	5.78	
ark-shell, *Anadara broughtonii*	4.86	1.05	0.75	0	0	0	6.66	Foot
	3.33	0.88	0.51	0	0	0	4.72	
abalone, *Haliotis discus hannai*	3.48	0.59	0.20	0	0	0	4.27	Adductor
	3.10	0.50	0.25	0	0	0	3.85	
crab, *Erimacrus ienbeckii*	5.87	1.75	0.29	0	0	0	7.91	Leg
	4.70	1.06	0.21	0	0	0	5.97	
crab, Paralithodes brevipes	3.51	1.64	0.26	0.16	0	0	5.57	Leg
squill, *Squilla oratoria*	3.84	1.24	0.94	0.50	0.46	0	6.98	Abdominal
prawn, *Pandalus hypsinotus*	6.61	2.09	0.94	0	0	0	9.64	Abdominal
	6.93	1.38	0.54	0	0	0	8.85	
carp, *Cyprinus carpio*	4.68	0.95	0.04	0	0	0	5.67	Dorsal
rainbow trout, *Salmo gairdnerii irideus*	5.84	1.06	0.54	0.22	0	0	7.66	Dorsal

$$\text{Fish:} \quad K(\%) = \frac{HxR+Hx}{ATP+ADP+AMP+IMP+HxR+Hx} \times 100$$

$$\text{Mollusk:} \quad K(\%) = \frac{HxR+Hx}{ATP+ADP+AMP+HxR+Hx} \times 100$$

$$\text{Crustacean:} \quad K(\%) = \frac{HxR+Hx}{ATP+ADP+AMP++IMP+AdR+HxR+Hx} \times 100$$

IMP: inosine monophosphate, AdR: adenosine, HxR: inosine, Hx: Hypoxanthine

Fig. 6 Definition of K value of muscle from marine animals.

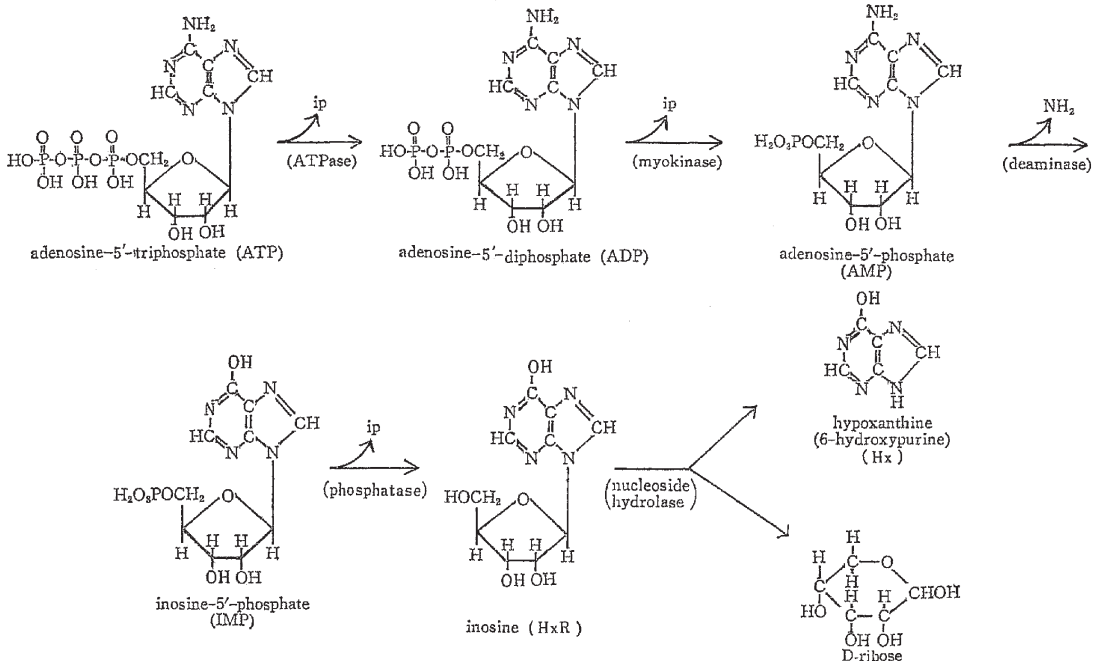

Fig. 7 Pathway of ATP breakdown in fish muscle and related enzymes

example, to emphasize the ratio of hypoxanthine to inosine, but sorting is time consuming.

The first report using K value to evaluate the freshness of fish and shellfish meat was published in 1959. The paper reported that the K values obtained from 17 test samples of frozen fish corresponded well to the record and the organoleptic freshness evaluation of the supplier. Discussion continued and the following K values are now used to evaluate freshness: 0 to 10%, living fish and fish that can be provided for "Arai"; 20% or below for "Sashimi"; 15 to 35% for fresh fish on the general consumer market; 40% or below for stewed fish; and 60% or below for processed materials such as minced fish meat (Surimi). Fish meat with a K value of 60% or more is in initial putrescence (Fig. 8). This is reasonable considering that actions of the enzyme group involved in ATP decomposition depend on the conditions of the fish and shellfish after death, particularly with regard to temperature history and time, although K value is considered to indicate how fresh the sample is. However, the quantity and activity of the enzymes involved in ATP decomposition differ with species, and may even vary in individuals of the same species. Thus, the speed of ATP decomposition and K value can vary between the cases. According to examinations into the relationship between K value and storage temperatures of plaice muscles, K value rises faster at higher temperatures (Fig. 9). However, the reaction patterns are not simple and the speed may vary with time. This is presumably caused by changes in biochemical conditions in

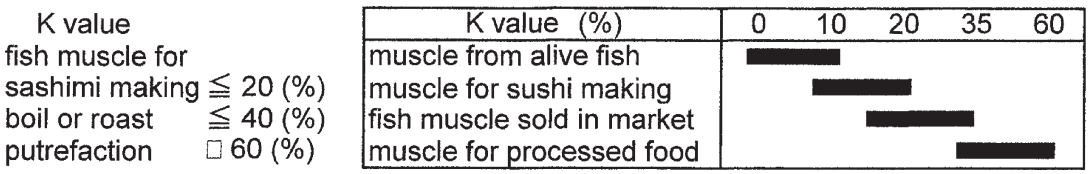

Fig. 8 Suitable K value of fish muscle for various utilizations. (E. Watanabe, 2004)

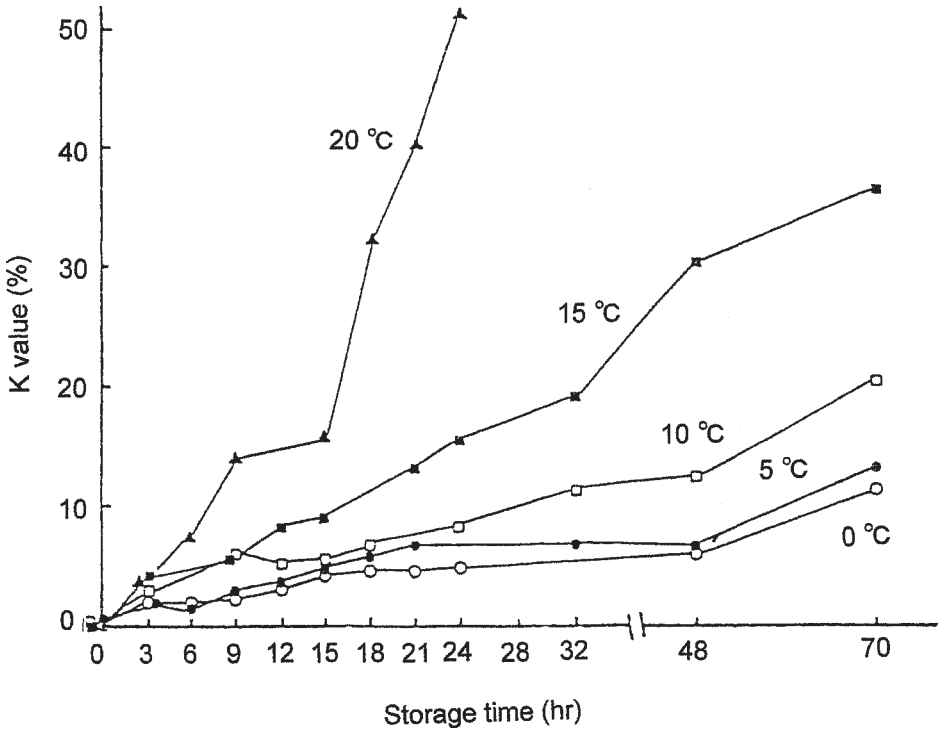

Fig. 9 Change in K value of plaice muscle during storage at various stored temperatures. (M Iwamoto *et al*, 1987)

the muscles and decreasing pH as ATP is decomposed. Comparing the changes in K value of 8 different fish and shellfish under ice storage (Fig. 10), the speed of increase and reaction patterns for K value significantly vary in each type of fish. The cause has not yet been determined, but it may be related to changes in activity level of the related enzyme group and in biochemical conditions. It has already been reported that nutrition, physiological conditions, circumstances of death, muscle type and storage temperature all affect the K value after death.

The loss of ATP in muscle and the increase in K value correspond to the end of muscle rigor. As described above, fish and shellfish muscles with K values of 20% or below are suitable for consumption as "Sashimi", and are at the initial stages of muscle rigor. For Japanese who eat raw fish and shellfish, such a relationship represents an extremely convenient discovery. However, examining changes in muscle rigor reveals that K value has a very complex relationship with storage temperature. For example, when red sea bream was immediately killed after capture and stored at either 0°C or 10°C, the lower temperature rapidly brought on muscle rigor and reduced ATP content when compared with the higher temperature (Fig. 3). As described above, this indicates that cooling contracture has occurred in the muscles. At 10°C, the muscles contract and become rigid when the endoplasmic reticula and mitochondria lose their capacity for Ca ions as ATP is lost. On the other hand, at 0°C, the membrane of the endoplasmic reticula and mitochondria is damaged, thus leading to leakage of Ca ions, despite the higher ATP content. As a result, the fish meat is contracted and rigor starts (the timing of rigor and ATP loss differ slightly). When plaice is stored at 0 to 20°C (Fig. 11), rigidity indices increase more slowly between 5 and 15°C, and the pattern of this increase becomes stepwise and complex. This is also an effect of cold shortening. It is known that leakages of Ca ions

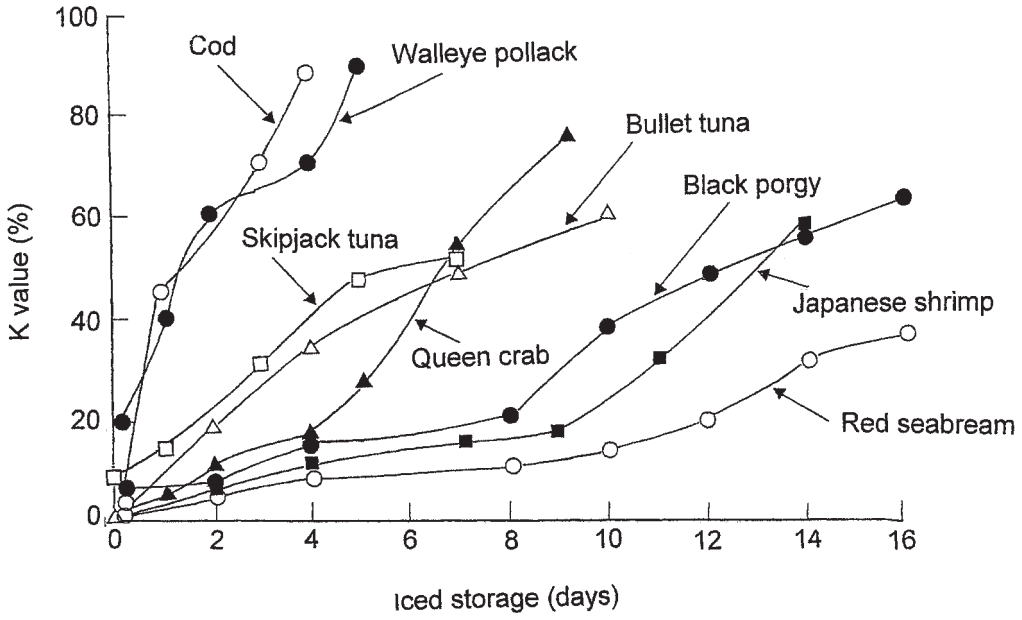

Fig. 10 Change in K value of various fish and crustacean muscles during iced-storage. (E Watanabe, 1998)

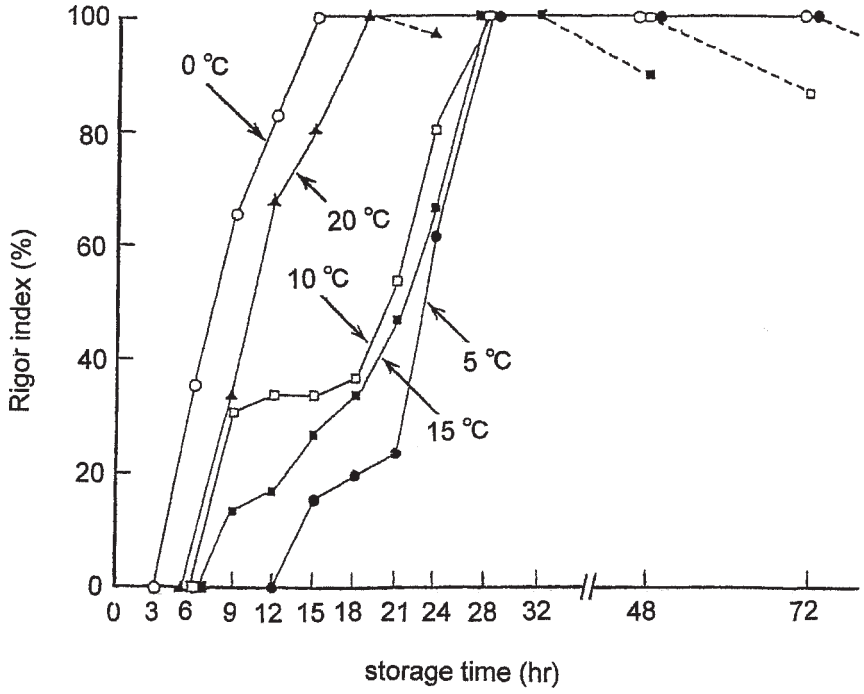

Fig. 11 Change in rigor index of plaice muscle during storage at various temperatures.

from the endoplasmic reticula and mitochondria are caused by the decreasing pH in the muscles. In scallop adductor muscles, the muscle becomes rigid when the pH goes below 6.5 and K value exceeds 20%, as ATP is lost (Fig. 12). It is speculated, however, that this rigidity is different in nature because it is caused by ATP loss and decreased pH.

In animal muscles, whether from livestock, fish or shellfish, ATP contained in the muscle is reduced during storage, and is lost when the muscles enter complete rigor. In exceptional cases, depending on storage temperature and pH, rigor is abnormally advanced by leakage of Ca ions from the endoplasmic reticula and mitochondria. Normally, decomposition of ATP is slow

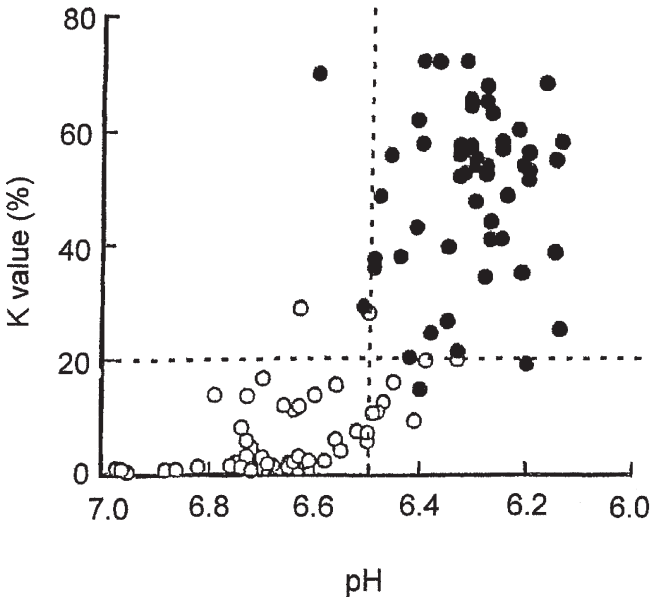

Fig. 12 Relation among pH, K value and hardness of scallop adductor muscle
●: soft, ○: hard

at low temperatures and increases in K value depend on storage temperatures. Decomposition is never faster at low temperatures. Accordingly, appropriate storage conditions for fish and shellfish must be determined by considering their use, with sufficient understanding of how ATP content, rigidity and K value are affected by the physiological conditions of fish and shellfish meat.

The protein content of fish and shellfish muscles changes relatively slowly, in comparison with muscle rigidity and K value. Protein is a large molecule and there are several indices for changing quality. We measured the gel formation potential and myosin Ca-ATPase activity of paste products and frozen minced fish, and compared these data with the K values of ice-stored fish (Fig. 13). Among three different types of fish, the K values rapidly increased in walleye pollack (*Theragra chalcogramma*), but increased slowly in white croaker (*Pennahia argentata*) and mackerel (*Scomber japonicus*). However, the Ca-ATPase activity in frozen minced fish sample declined fastest in the mackerel, and relatively slowly in the white croaker and walleye pollack. The breaking strength of the paste products ("Kamaboko") prepared from frozen Surimi under certain conditions also declined fastest in mackerel, and slowly in the white croaker and walleye pollack. This indicates that even though the muscles were extremely fresh (K value was 20% or less), if mackerel is processed into frozen Surimi, the quality is relatively inferior and the gel formation ability actually deteriorates. We have confirmed that this is caused by acid denaturation of myosin owing to decreased pH along with the rapid production of lactic acid in the mackerel muscles. In contrast, the walleye pollack shows a drastic increase in K value. Even when K value is around 60%, which is corresponding to initial putrescence, denaturation of protein is limited and the gel formation ability is retained. As described above, this clearly shows that an increase in K value is caused by actions of several enzyme groups, and proceeds separately from protein denaturation. Similarly, the degree of microbial contamination in the stored fish meat and that of protein denaturation indexed by myosin ATPase activity are

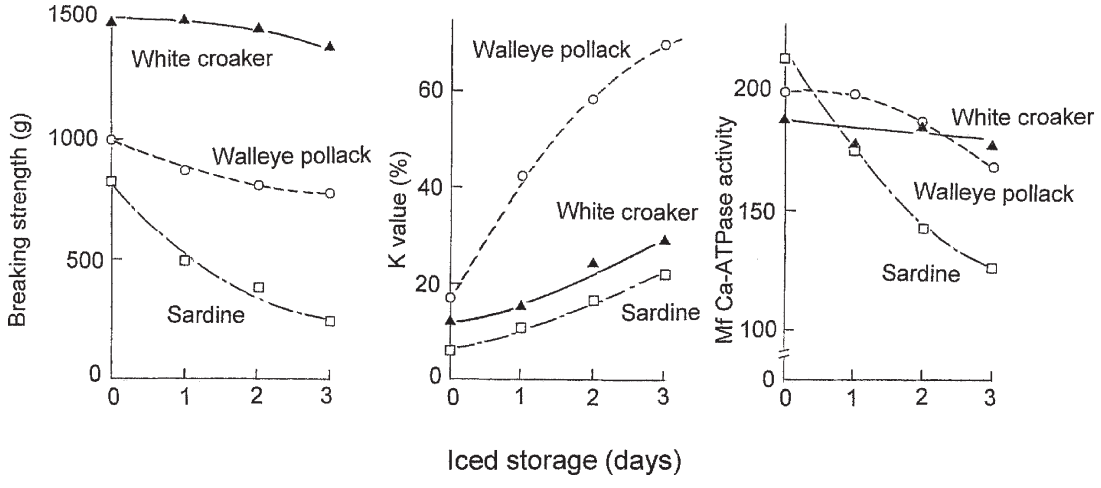

Fig. 13 Changes in K value, myofibrillar Ca-ATPase activity and gel formation of muscle from three fishes during iced-storage
pH of muscle is 5.7 (sardine), 6.8-7.2 (white croaker and walleye pollack).

completely unrelated.

Generally, K value is measured by high-speed liquid chromatography using an ion-exchange resin. It is also measured by thin layer chromatography with ECTEOLA-cellulose as a carrier. Recently, fast and simple measuring methods have been successfully devel oped and sold, including a biosensor method using decomposition of ATP-related compounds and the enzyme groups related to oxidation-reduction, and enzyme electrode (by EAC Corporation), as well as freshness test paper on which related enzymes are attached and dried (also by EAC Corporation).

4. Freshness and quality evaluations of fish and shellfish.

The terms "freshness" and "quality" are often used interchangeably when applied to assessments of fish and shellfish. However, this is misleading as these qualifies actually refer to different characteristics.

The term "freshness" of fish and shellfish essentially refers to the quality of raw fish fresh and shellfish, but is not used to describe that of processed foods, such as fish paste products ("Kamaboko") and dried products ("Himono"). In the context of processed foods, the term "freshness" would be employed as an indicator of the storage history of a product following its production. In effect, it would be equivalent to an "expiry date" and would primarily serve as an indicator of food safety. In fact, the K value (degradation product of ATP), which is used to assess the freshness of raw fish and shellfish, cannot be applied to fish paste and dried fish products as ATP is degraded and ooze out during processing and production.

The K value for fish and shellfish reflects the rate of ATP degradation as a percentage of degradation products. However, ATP degradation depends on the activity of a group of enzymes in muscle tissue and the rate of the reaction is determined by environmental temperature and time. In other words, freshness only reflects the temperature history of the fish and shellfish since they died.

Changes in the muscle tissue of fish and shellfish following death are not limited to ATP degradation, and a wide variety of changes have been characterized. The most obvious biochemical changes include the enzymatic production of amino acids, amines (histamine, etc.) and lactic acid by glycolysis (associated with decreased pH), as well as the denaturation of muscle proteins, and the oxidation of lipid components (polyunsaturated fatty acids). Physical changes, such as stiffening and softening of muscle tissue, changes in the water retention capacity of tissues and a variety of other manifestations also occur. In addition, after landing, fish and shellfish may become contaminated by a variety of microorganisms during storage, transport and processing. Consequently, considerable emphasis has recently been placed on the need to ensure the hygiene and safety of fish and shellfish, as well as the numerous processed foods derived from them.

The aforementioned physico-chemical changes all progress at different rates and depend on the species of fish and shellfish, physiological conditions, catch and storage methods, and other factors. For example, the muscle proteins of Japanese sardines (*Sardinops melanostictus*) are rapidly denatured after death due to the accumulation of high concentrations of lactic acid in muscle tissue. In Antarctic krill (*Euphausia superba*), damage to the digestive tract, which is easily damaged during landing, results in the exudation of digestive proteases and consequent digestion of the muscle tissues. In sharks, the urea present in muscles promotes the denaturation of muscle protein. There are thus numerous factors that have a marked effect on the rate of muscle protein deterioration, and these changes may exceed the rate of increase in the K value. Since similar changes may also occur in a variety of other substances (and properties), both within and outside the muscle tissue of fish and shellfish, the analysis of these reactions from the standpoint of kinetics is necessary.

Conversely, the rate of these changes in terrestrial animal muscle tissue generally occurs very slowly after death, and ATP degradation, changes in muscle hardness, and the denaturation of protein are all relatively slower. This has resulted in active research on processing technology for promoting the softening of muscle tissue. Processing technologies for aging, which soften the meat and enhance the flavor through the production of amino acids and inosinic acid, are currently well established. However, except for special cases, these technologies are rarely applied to fish and shellfish. Similarly, the K value has rarely been used as a freshness indicator for livestock meat of terrestrial animals.

Japanese food culture incorporates the consumption of fresh fish and shellfish which are eaten raw as "Sashimi", and "Sushi", etc. Accordingly, technology and knowledge for maintaining the firmness of muscle tissue is highly valued and has led to the widespread use of the K value,

which very closely reflects the firmness of muscles, as an indicator of freshness in Japan. In addition, in the high-protein foods such as fish paste products and dried products, which have been mass-produced in Japan, the properties of the muscle protein and their natural changes are considered to have a marked effect on quality. For this reason, a kinetic analysis of denaturing reactions of muscle protein is required for use in the development of indicators for quality management. In the case of fish paste products, the use of large quantities of superior heat-induced gel-forming protein in the frozen Surimi to form a proteinaceous gel structure that confers the texture of "Kamaboko" have become well accustomed. However, the properties of the proteins in frozen Surimi that confer good heat-induced gel-forming ability and their contribution to the texture of fish paste products are still not adequately clarified. This is a major project currently being undertaken by the authors' research group.

Furthermore, future's research topics will include finding appropriate indicators for evaluating food within nutritional, healthy and safety contexts, as well as developing technologies for the establishment of food quality standards.

References

1. On Post-mortem Changes

A. L. Lehninger (1968): Bioenergetics, 2nd edition. W. A. Benjamin Inc., Menlo Park, California, U.S.A, 244pp.

M. Suyama and S. Kounosu (1988): Suisan Shokuhingaku. Kouseisha-Kouseikaku, Tokyo, 342pp.

Yamanaka, H. (2002): Relation between Post-Mortem Biochemical Changes and Quality in the Muscles of Fish and Seashell. Nippon Suisan Gakkaishi, **68**, 5-14 (in Japanese).

2. On Evaluations of Freshness and Quality

E. Watanabe (1995): Measurement of Freshness and Assurance of Seafood Quality. Kouseisha-Kouseikaku, Tokyo, 126pp.

3. On Estimation of K value as Freshness Index

Saito, T. *et al*. (1957): Slow Freezing of carp Muscle and Inosinic Acid Formation. Nature, 179, 820.

Saito, T. *et al*. (1959): A New method for Estimating the Freshness of Fish. Bulletin of the Japanese Society of Scientific Fisheries, **24**, 749-759.

Arai, K. (1966): Acid-soluble Nucleotides in Some Marine Invertebrates. Bulletin of the Japanese Society of Scientific Fisheries, **32**, 174-179.

T. Saito, H. Uchiyama et al. (1974): Experimental Book of Fisheries Biochemistry and Food Science. Kouseisha-Kouseikaku, Tokyo, 509pp.

M. Abe and N. Uchida (2004): Current Situation and Problem for the Control of Fish Quality and Freshness. Kouseisha-Kouseikaku, Tokyo, 157pp.

E. Watanabe (2004): Fishery Food Design. Seizando Shoten, Tokyo, 236pp.

J. Nakazoe and H. Yamanaka (2004): The Use of High Technology for the Control of Fish Quality and Freshness. Kouseisha- Kouseikaku, Tokyo, 147pp.

E. Watanabe, N. Kato, H. Ohkuma, and N. Hamada (2007): Freshness of Fish. Seizando Shoten, Tokyo, 112pp.

要　旨

魚介類の鮮度と品質（追補）

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魚介類の鮮度を評価するため，これら筋肉中の ATP の貯蔵に伴う分解生成物を定量した．そして K 値を B/A×100 で表したが，B はイノシン＋ヒポキサンチンの合計量，A は ATP 関連化合物の合計量である．水生動物の筋肉の死後硬直の開始と，それに続く軟化は陸上動物のそれよりも著しく早く起こるが，筋肉中の ATP 含量は，筋肉の死後硬直の変化と強く関わっていることが知られている．結果的に，筋肉の鮮度を K 値を用いて評価する方法が我が国で人気を克ち得たのは，これらの動物の筋肉切片を硬い状態で“さしみ”や“すし”に使うのが好ましいからである．

筋肉の加工食品の種類によっては，K 値に加えて，さらに適切な評価指標，たとえば官能的，物理的及び微生物的試験を追加しなければならない．この理由は多くの指標による方がより納得できる製品の品質評価ができるからである．

キーワード：魚介類，鮮度，品質，K 値