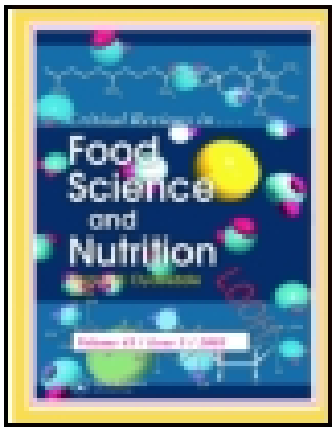

Critical Reviews in Food Science and Nutrition
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/bfsn20

The Importance of ATP-related Compounds for the Freshness and Flavor of Post-mortem Fish and Shellfish Muscle: A Review

Hui Hong[ab], Joe M. Regenstein[b] & Yongkang Luo[a]

[a] College of Food Science and Nutritional Engineering, China Agricultural University, P. O. Box 112, Beijing 100083, Peoples Republic of China

[b] Department of Food Science, Cornell University, Ithaca, NY 14853-7201, USA

Accepted author version posted online: 26 Jun 2015.

The Importance of ATP-related Compounds for the Freshness and Flavor of Post-mortem Fish and Shellfish Muscle: A Review

Hui Hong[a, b], Joe M. Regenstein[b], Yongkang Luo[a*]

[a] College of Food Science and Nutritional Engineering, China Agricultural University, P. O. Box 112, Beijing 100083, Peoples Republic of China

[b] Department of Food Science, Cornell University, Ithaca, NY 14853-7201, USA

[*]Corresponding author. Tel.: +86-10-62737385; Fax: +86-10-62737385

Email: luoyongkang@263.net; luoyongkang@cau.edu.cn (Yongkang Luo)

Abstract:

ATP degradation is one of the most important biochemical changes in the post-mortem muscle of fish and shellfish. This process has long been recognized as an accurate way to evaluate freshness of fish and shellfish product. This review updates and condenses the overall history and recent advances in understanding the role of ATP-related compounds in post-mortem fish and shellfish muscle including a discussion of key analytical methods, their use as a freshness indicator, their roles in flavor enhancement, the factors affecting their transitions, and the possible mechanisms responsible for their impact on flavor and freshness. Moreover, some challenges and future directions for research regarding ATP-related compounds in fish and shellfish flavor and freshness are presented. With increasing consumer demands for fresh products with extended shelf life, understanding the relationships between ATP-related

compounds and their involvement in the freshness and umami taste is a prerequisite for assuring the high quality of fish and shellfish.

Abbreviations: AD, AMP deaminase; **ADK**, Adenylate kinase; **ADP**, Adenosine diphosphate; **AMP,** Adenosine monophosphate; **AP**, Alkaline phosphatase; **ATP**, Adenosine triphosphate**; Cr,** Creatine; **CrK**, Creatine kinase; **GMP**, Guanosine monophosphate; **HPLC**, High-performance liquid chromatography; **HPP,** High-pressure processing; **Hx,** Hypoxanthine; **HxR or Ino,** Inosine**; IMP,** Inosine 5′-monophosphate; **MSG**, Monosodium glutamate; **MTT**, 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide; **NP**, Nucleoside phosphorylase; **NT**, 5'-Nucleotidase; **PCr**, Creatine phosphate; **polyp, P**olyphosphate; **PPT**, Phosphotransferase; **UA,** Uric acid; **Xa,** Xanthine**; XOD or XOx**, Xanthine oxidase;

Keywords: rigor mortis, biosensors, freshness indicators, umami, hypoxanthine

INTRODUCTION

Fish and shellfish are extremely perishable, compared to other muscle foods. Fish or shellfish will enter into *rigor mortis* just a few hours after death. The *rigor mortis* is a process where a fish loses its flexibility due to the stiffening of its muscle. The increasing muscle tension during the onset of *rigor mortis* is correlated with a decrease in ATP-related compounds. Significant breakdown of ATP will start within 6 h of catch if the fish or shellfish are at high ambient temperatures (Lakshmanan et al., 1996).

ATP, the "energy currency" of the cell, is a nucleoside triphosphate that functions as a coenzyme to transport chemical energy within cells to various intracellular spaces and to enzymes that use it to carry out their function as part of metabolic processes. The majority of this energy is used by muscle cells to do mechanical work and to synthesize proteins, urea, and other metabolic intermediates. Thus, living organisms try to maintain a relatively constant amount of ATP within each cell. However, during the post-mortem storage of vertebrate fish or of shellfish, ATP in muscle tissue degrades in a series of biochemical reactions commonly represented by the sequence shown in Figure 1.

IMP, as one of ATP breakdown compounds, is associated with the umami taste (a pleasant savory taste) of fish and shellfish. In the food industry, IMP and its salts are widely used as flavor enhancers. They are mostly added to soups, sauces, and seasonings for the intensification and balance of meat flavor. However, more efforts should be made to maintain the IMP level in

fresh fish and shellfish in response to consumer demands for freshness, safety and taste.

Despite the existence of an extensive literature on the measurement of ATP-related compounds for freshness of fish, published reviews on the role of ATP-related compounds in post-mortem fish muscle are limited. An early, brief review published in 1962 compared the findings at that time with respect to ATP and glycogen in fish and mammalian skeletal muscles (Tomlinson and Geiger, 1962). Another review published in 1966 provided a concise summary of the post-mortem changes in glycogen, nucleotides, sugar phosphates, and sugars in fish muscles (Tarr, 1966). Most recently, Howgate (2006) modeled the kinetics of the degradation of IMP in some species of fish during chilled storage. Delbarre-Ladrat et al. (2006) reviewed the proteolysis and disorganization of the myofibrillar structure in post-mortem fish. However, no review has been published on the changes of ATP-related compounds and their close relationship with freshness and flavor of post-mortem fish.

Therefore, the purpose of this review is (1) to examine the current literature on ATP-related compounds in post-mortem fish and shellfish with an emphasis on their impact on freshness and flavor, and (2) to indicate some promising ways to control or delay the process of ATP degradation.

DEGRADATION MECHANISMS

Post-mortem changes in fish muscle can be briefly summarized as follows: catching - *rigor mortis* – resolution of *rigor mortis* - autolysis – spoilage (Hamada-Sato et al., 2005). The actual

rate of these reactions will be affected by the fish species, their health and composition at the time of harvesting, water temperature at the time of catching, the history of the fish starting from the time of interaction with different catching procedures, and the subsequent history of handling.

ATP degradation is an integral part of this process of post-mortem changes in fish and shellfish. In the living state, all organs and systems within the body cooperate to maintain an internal environment so that each component can perform its function efficiently. When this stable interior environment is disrupted, the supply of oxygen to the muscle tissue is interrupted because the blood is no longer being pumped by the heart, and the enzymes responsible for ATP degradation are no longer inhibited (Huss, 1995).

The normal energy-generating metabolism of muscle is provided by the phosphagen system (ATP-PCr), glycolytic system and the aerobic oxidation system in living mammals, birds, and fish (Proctor and McLoughlin, 1992; Scheffler et al., 2011). Following removal from water most fish and shellfish cannot obtain oxygen from the environment. Energy production from ingested nutrients is greatly restricted since no oxygen is present for normal respiration. **Figure 1** illustrates the normal pathway for most teleost fish for the synthesis of ATP to become available for use by cells, and to provide muscles with the energy they need.

In living fish or shellfish, ATP requirements for muscles are normally met by the aerobic oxidation system. During vigorous exercise, however, such as when escape behavior becomes

necessary, ATP may be depleted, which causes leading the burst (white) muscles becoming nominally anaerobic and to produce some lactic acid. However, for a post-mortem muscle to generate ATP, it must use the two remaining pathways: the creatine phosphate (PCr) pathway and/or the anaerobic glycolysis pathway. The PCr pathway uses the creatine kinase (CK) in muscle of higher animals to convert ADP and PCr to ATP and Cr. In most cephalopods or terrestrial red crabs, *Gecarcoidea natalis* (Morris and Adamczewska， 2002), however, the ATP regeneration is based on a transfer pathway from arginine phosphate to arginine.

In both types of animals, anaerobic glycolysis can also occur (Figure 2). Muscle glycogen is broken down and metabolized through glycolysis to pyruvate. The pyruvate is then reduced to lactic acid by lactate dehydrogenase when oxygen is limited. The nutritional status of the fish and the amount of stress and exercise encountered before death will have an effect on the amount of stored glycogen available and, consequently, on the ultimate post-mortem pH. Glucose can also enter the glycolysis pathway after being phosphorylated by hexokinase, but this reaction contributes very little to the post-mortem formation of ATP in the absence of blood circulation.

With the gradual depletion of PCr and glycogen, ATP degrades rapidly. At the same time, the breakdown of ATP results in an increasing amount of ADP, which is the substrate for the adenylate kinase reaction that produces 1 ATP and 1 AMP from 2 ADP. Consequently, AMP increases and is then deaminated to IMP, which accumulates in many species of post-mortem fish (Massa et al., 2005). IMP is the main nucleotide present in most fish species post-mortem,

whereas AMP remains the major nucleotide in crustaceans (Mendes et al., 2001). The IMP is further degraded to Ino and the disappearance of IMP has been connected to the loss of freshness in some fish species (Saito et al., 1959). Ino is then transformed to Hx by the nucleoside phosphorylase (NP) enzyme. The Hx is finally converted to Xa, uric acid and other ring cleavage products by the developing spoilage microflora (Mohan et al., 2009).

As the breakdown of ATP exceeds the rate of synthesis, less ATP is available to "relax" the muscle, leading to the loss of the ability of myosin to disassociate from actin. When ATP is depleted, cross bridges between myosin and actin cannot be broken, and the muscle is relatively inextensible, i.e., in *rigor mortis* (Scheffler et al., 2011).

ANALYTICAL METHODS

Sound analytical methods are indispensable when studying the ATP degradation mechanisms. There are two reasons for the determination of ATP-related compounds in post-mortem fish and shellfish muscle that are relevant to this review: the first is the potential of using them as freshness markers; the second is their relation to sensory attributes. Various methods have been developed for the analysis of ATP-related compounds, including reverse-phase HPLC with or without creating an ion pair, capillary electrophoresis, biosensor assays, radioimmunoassays, and bioluminescence. Before using these techniques, the ATP-related compounds are generally extracted from the muscle matrix.

Extraction of ATP-related compounds

Typically, small tissue samples are immersed into liquid nitrogen to avoid further ATP-chain degradation prior to extraction. The classical extraction method starts with homogenization of a sample with cold perchloric acid solution, which is then centrifuged at high speed. The supernatant is collected and neutralized to pH 6.4–6.5 using a KOH or NaOH solution. The resultant solution is filtered through a 0.2-0.45 μm membrane filter and stored at −20 to -70 ºC until further analyzed (Ryder, 1985; Song et al., 2012). However, a standard extraction process has not been established and the use of perchloric acid is extremely hazardous and can cause environmental and health damage.

Many researchers realized that the tedious acid digestion procedure was not necessary for measurement of nucleotides and nucleosides. They used distilled water or phosphate buffer (pH 8.2) to extract ATP-related compounds (Lawal and Adeloju, 2012; Nanjyo and Yao, 2002; Thandavan et al., 2013; Devi et al., 2013). A heating process has also been introduced to extract ATP-related compounds. For instance, fish muscle can be heated in a microwave oven (500 W, 15 s), and then pressed to collect the nucleotide extracts (Okuma and Watanabe, 2002).

Chromatographic Methods

Chromatographic methods are widely used for the simultaneous analysis of nucleotides in aquatic food samples. The nucleotides can be separated by ion-exchange chromatography (Saito et al., 1959), HPLC (Özogul et al., 2000) and capillary electrophoresis (Soga and Imaizumi, 2001) (Table 1).

The original analysis for ATP-related compounds was based on ion-exchange chromatography (Saito et al., 1959), which is time-consuming and unstable. At present, the most frequently used method for analysis of ATP-related compounds is HPLC (Kuda et al., 2008; Özogul et al., 2000). Typically, components are separated using reverse-phase buffers or ion-pairing methods. The addition of an ion pair to the mobile phase greatly improves the separation by increasing the retention time of charged molecules (ATP, ADP, and AMP). The ion pair reagent should have a positive ion with a hydrophobic characteristic to improve the affinity of the charged molecules to the stationary phase, due to the negative charge of the phosphorylated groups of the ATP-related compounds. Thus, either tetrabutylammonium hydrogen sulfate or phosphate are the ion pair compounds most often used (Zur Nedden et al., 2009). Zur Nedden et al. (2009) also showed that ion-pair HPLC could be done rapidly and had a high sensitivity and extraction efficiency. However, the disadvantages of the ion-pair approach to the HPLC are the short service life of the column, the complicated procedure to assure the accuracy of the quantitative analysis, and the fact that the method is relatively more expensive (Khlyntseva et al., 2009).

Ryder (1985) reported on an HPLC method that uses a simple reverse-phase separation with a commercially available column. This method provides more rapid, quantitative analysis of ATP and its breakdown products compared with the previous methods that use ion-exchange chromatography. This method has been widely adopted to determine ATP-related compounds in

post-mortem fish and shellfish (Aubourg et al., 2005; Hong et al., 2012b; Li et al., 2013; Mendes et al., 2001; Özogul et al., 2008; Rodríguez et al., 2006).

Mora et al., (2010) developed a new HPLC method based on hydrophilic interaction chromatography for the simultaneous analysis of ATP, ADP, AMP, IMP, Ino, Hx and adenine dinucleotide. The results obtained using hydrophilic interaction chromatography largely agreed with those obtained using ion-pair HPLC. Thus, hydrophilic interaction chromatography is also a reliable alternative for isolating and quantifying nucleotides in complex matrices such as meat, and presumably fish and shellfish, although it does not seem to have been used for this purpose to date.

Capillary electrophoresis (CE) is a powerful analytical technique that can give faster results and provide high-resolution efficiency with minimal sample volume and buffer consumption. Nguyen et al. (1990) first applied CE to separate and quantify the IMP, Ino, and Hx in fish tissue. The three major nucleotide compounds in fish fillets, IMP, Ino, and Hx were distinctively separated. Thereafter, Luong et al. (1992) combined CE and an immobilized enzyme procedure to monitor degradation of IMP, Ino, and Hx in Atlantic cod (*Gadus morhua L.*), salmon (*Salmo salar L.*) and trout (*Oncorhynchus mykiss*). These two procedures worked well for all three species. In 1998, Geldart and Brown reviewed the methods used for the optimization of nucleotides by CE and its use in analysis of nucleotides in biological matrices. They felt that CE could represent an attractive alternative to HPLC for the analysis of nucleotides. Most recently,

Tomoyoshi Soga et al. (2007) determined the nucleotides in *Escherichia coli* using a pressure-assisted CE combined with electrospray ionization mass spectrometry. This has become a good way to analyze metabolites in biological samples. CE analyses are usually faster than HPLC analyses and require smaller sample volumes, thus making single cell analysis possible (Tseng et al., 1994). However, CE has only had limited use in determining ATP-related compounds in fish or shellfish samples because of its relatively poor reproducibility and the higher detection limit when compared with HPLC. Another problem is that only a few companies are producing CE equipment, compared to HPLC, thus the equipment is more expensive and less money has been spent on the development of equipment.

BIOSENSORS

Enzyme Sensors

Enzyme sensors, together with immunosensors for pathogenic bacteria and pesticides, are the main biosensors employed at this time in food analysis (Venugopal, 2002). An enzyme sensor is an analytical device that combines enzymes with a transducer to convert the biological response into a measurable electrical signal such as current, potential or voltage. These signals are proportional to the target analyte concentration if the system is working properly.

The reported enzymes commonly used in the determination of ATP-related compounds were AMP deaminase (AD), 5'-nucleotidase (NT), alkaline phosphatase (AP), nucleoside phosphorylase (NP), and xanthine oxidase (XOD). The role of each enzyme in degrading

ATP-related compounds is shown in the following reaction scheme, where Pi is inorganic phosphate:

$$AMP \xrightarrow{AD} IMP \xrightarrow{NT/AP} Ino \xrightarrow{NP,Pi} Hx \xrightarrow{XO,O_2} Xa \xrightarrow{XO,O_2} UA \quad (1)$$

These enzymes were immobilized on support matrices (cellulose triacetate, aminopropyl glass, chitosan, etc.) by physical and/or chemical methods and attached to oxygen electrodes (Carsol and Mascini, M., 1998; Okuma and Watanabe, 2002; Park and Kim, 1999) which then responded to changes in oxygen concentration due to the action of the enzyme.

Measurements of ATP-related compounds by enzyme sensors were based on the principle that the current output of the oxygen electrode decreased due to the consumption of oxygen, when Hx is oxidized to Xa and finally uric acid (UA) by xanthine oxidase (XOD).

This system has been successfully applied for quality assessment of sea bass, saurel (*Trachurus japonicus*), mackerel (*Sxcomber japonicus*), yellowfish (*Seriola quinquediata*), gilthead bream (*Sparus aurata*), yellowfin tuna (*Thunnus albacares*), common carp (*Cyprinus carpio*) and mirror carp (*Cyprinus carpio var. specularis*) (Carsol and Mascini, M., 1998; Karube et al., 1984; Nanjyo and Yao, 2002; Okuma and Watanabe, 2002; Park and Kim, 1999; Volpe and Mascini, 1996; Watanabe et al., 1984).

Watanabe et al., (2005) developed another system for fish quality evaluation that combined enzymes with a colorimetric sensor. The system is based on the principle that the freshness of sashimi, which is judged by using the K_I value (See Table 2), can be determined from the degree

of color change of thiazole blue (MTT: 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide). The color variation was induced by the redox reaction of MTT together with the oxidation of Hx by XOD. A good correlation was obtained between the K_I value and the remaining days of shelf-life as determined from the color, where the end of shelf-life was based on reaching the limit of the K_I value (22%).

Lawal and Adeloju (2012) developed a XOD potentiometric biosensor for HX based on using a ferrocene carboxylic acid modified electrode. A linear response to Hx was obtained by the sensor in the concentration range of 5 to 20μM (r= 0.998) and was successfully used for the determination of Hx in sword shark (*Pristis pectinata*). Niu and Lee (2000) designed an amperometric biosensor for Hx based on a sol–gel technique. This technique took advantage of the ability to encapsulate proteins and enzymes. The sensors had a shelf life of more than 2 months, if stored at 4°C.

Most recently, a nanobiosensor with a nano-interface (Fe_3O_4) using XOD was developed to detect the levels of Xa in fish samples (Thandavan et al., 2013). The iron oxide (Fe_3O_4) nanoparticles were synthesized by co-precipitation of Fe^{2+} and Fe^{3+}aqueous solutions with the addition of ammonium hydroxide. The XOD enzyme was then covalently linked to the Fe_3O_4 nanoparticles. Thereafter, the XOD/nano-Fe_3O_4 was tagged on the surface with a working gold (Au) electrode. The Xa content was determined based on the current produced by the reaction between the XOD–Fe_3O_4 modified gold electrodes and the fish extract. This biosensor had a

lower detection limit (2.5pM) and a quicker response time (less than 2 sec) compared with various other biosensors tested: biosensor made with XOD/nano-NiO (Yadav et al., 2012), XOD/ZnO/pyrrole/Pt (Devi et al., 2011), XOD/ZnO/chitosan/multiwalled carbon nanotube/polyaniline (Devi et al., 2012), or XOD/ silver nanoparticles (AgNPs) /l-Cys/Au (Devi et al., 2013).

Although biosensors have shown their utility in some applications, such as in clinical, veterinary, environmental, agricultural, and industrial processing areas, the applications of biosensors in the food industry is still in its infancy mainly due to the challenges of enzyme immobilization and/or sample preparation for analysis. Additionally, the use of commercial kits is limited because of the relatively short lifetime of these sensors due to enzyme denaturation over time. Roberts et al. (1991) also claimed that the most serious drawback of the enzymatic method is that XO shows enzyme inhibition at high substrate concentrations.

Other Sensors

A potentiometric sensor has been developed by Barat et al. (2008) to measure IMP, Ino, and Hx so that it is possible to calculate the K_I index (See Table 2). This paper compared a conventional HPLC system for measurement of IMP, Ino, and Hx, with a potentiometric sensor coupled with gold (Au) and silver (Ag) electrodes. A least-squares statistical method was used to obtain the various correlation equations for the electric potential using the electrodes and some physicochemical, microbiological, and biochemical analyses of sea bream. Excellent correlations

were obtained for IMP, Ino, Hx, and K_I values determined using the sensor signals and the chromatographic analysis. This fast, low-cost, and non-destructive potentiometric method could be applied for *in situ* and on-site fish quality monitoring over a wide range of situations. A similar study done by Gil et al. (2008) used principle component analysis and neuronal network analysis to validate the potentiometric measurements of IMP, Ino, and Hx. Results support the feasibility of using this potentiometric system as a practical, easy, rapid, and effective assessment tool to measure fish freshness.

Although biosensors have been shown to be able to evaluate fish freshness effectively, a portable analyzer is still needed for routine on-site measurement of the K or K_I value (See Table 2). Itoh et al. (2013) fabricated an electrochemical microfluidic device with two sensing sites in the upper and lower streams of a flow channel to measure the K value as a means of evaluating the freshness of fish. The K values obtained using this device and those obtained by HPLC were in close agreement.

Other Methods

A radioimmunoassay for Hx was described by Roberts et al. (1991). A Suffolk Cross sheep was used to prepare anti-Hx antibodies. The Hx was detected based on its specific affinity to the corresponding antibodies. Results showed that the radioimmunoassay achieved a more rapid throughput of fish extracts, but it requires special precautions and licensing because radioactive substances are used.

Bioluminescence is the production and emission of light by a living organism, which occurs widely in marine vertebrates and invertebrates, as well as in terrestrial invertebrates, including fireflies. Kaminishi et al. (1992) detected the ATP content in fish muscle by measuring the intensity of emitted light using luciferase obtained from the American firefly. The bioluminescence results almost match those of the column chromatography. Later, Tanaka et al. (2001) developed a bioluminescence method for detecting AMP in bonito (*Sarda sarda*) extract by using polyphosphate (polyP)–AMP phosphotransferase (PPT) and adenylate kinase (ADK) from *Acinetobacter johnsonii* strain conjugated with firefly luciferase. The results showed that this method was able to detect AMP in food residues with a high sensitivity.

APPLICATION OF ATP-RELATED COMPOUNDS IN FISH AND SHELLFISH

Use of ATP-related Compounds as a Freshness Indicator

The fresh quality of muscle foods is highly correlated with the biochemical changes taking place during the post-mortem period. Among the chemical methods for measuring freshness, the concentrations of ATP and its breakdown products, ADP, AMP, IMP, Ino, and Hx, are used extensively to calculate a number of different specific indices of freshness in a wide variety of fish before bacterial spoilage commences. These freshness indicators are derived from measurements of the relative concentration of ATP and its breakdown products. They are summarized in Table 2. The *K* value was originally defined by Saito et al. (1959) and has been

one of the most effective indicators of the freshness of fish. Although many chemical methods can be employed to evaluate fish freshness, including moisture measurement, volatile compounds measurements, protein changes, and lipid oxidation (Cheng et al. 2013), ATP-related compounds often give the best results in the time frame before significant microbial spoilage occurs. Hamada-Sato et al. (2005) indicated that the quality of raw fish"sashimi" could be judged based on the *K* value rather than histamine. However, a shortcoming of the *K* value as a freshness index is its dependence on species, seasons, handling conditions, and methods of kill (Olafsdóttir et al., 1997). Saito et al. (1959) recommended that fish products with *K* values lower than 20% be categorized as very fresh, those with a *K* value less than 50% as moderately fresh, and those with *K* values higher than 70% as not fresh. However, Ehira and Uchiyama (1974) and Lin and Morrissey (1994) proposed that the rejection value based on the *K* value be closer to 60% for products that will be processed. A *K* value of 20% has been defined by Japanese researchers as the limit for raw fish ("sashimi" grade) consumption.

Given that several studies suggested that ATP, ADP, and AMP dropped rapidly and IMP increased sharply at 0 ºC 24 h post-mortem, Karube et al. (1984) simplified the *K* value by creating the K_I value (see Table 2), which excluded ATP, ADP, and AMP from being a part of their index of freshness quality. Hamada-Sato et al. (2005) noted that the K_I value might be more appropriate for fish freshness evaluation. However, according to Nanjyo and Yao (2002), Kyrana and Lougovois (2002), and Cheng et al. (2013), the K_I values were only useful for monitoring the

early stages of stored fish changes, but could not be used to determine the loss of acceptability or the end of storage life, which was generally due to microbial spoilage. Additionally, the K_I value could be easily determined by biosensors using an XOD probe based on a hydrogen peroxide electrode (Volpe and Mascini, 1996), a metallic potentiometric electrode (Barat et al., 2008; Gil et al., 2008), or an amperometric electrode (Ghosh (Hazra) et al., 1998) without separating each single ATP-related compound using HPLC. Watanabe et al., (2005) indicated that the K_I value corresponding to consumable sashimi was less than 22%, as derived from conventional sensory evaluation.

The *H* value was used by Luong and others as an index of freshness quality (Luong et al., 1992; Mohan et al., 2009; Özogul et al., 2010b; Özogul et al., 2006; Park and Kim, 1999; Scherer et al., 2006; Song et al., 2012). For those fish where the breakdown of IMP occurs gradually, the *Fr* value (or IMP ratio) may be an applicable indicator of freshness. The *G* and *P* values are reported to be the most useful with lean fish (Shahidi et al., 1994). In fatty fish, however, factors such as the development of rancidity may render the product unacceptable before meaningful *G* and *P* values can be acquired (Alasalvar et al., 2002).

The accumulation of Hx in fish and shellfish is apparently responsible for the progressive loss of a desirable flavor, resulting in a bitter taste. Hx can be formed by the autolytic breakdown of nucleotides, but it can also be formed by bacteria, including *Pseudomonas* spp., S. *putrefaciens*, and P. *phosphoreum* (Huss, 1995). Hx can only be detected when the population of

spoilage bacteria exceeds 10^6 cfu/g tissue. Hx by itself has been widely used as a freshness indicator in fish (Jones et al., 1964; Lawal and Adeloju, 2012; Spinelli et al., 1964). Most recently, measurements of Hx have become a promising freshness indicator using both amperometric and potentiometric biosensors (Lawal and Adeloju, 2012; Thandavan et al., 2013). However, there is a pressing need for new methods or strategies for fabrication of more robust and more sensitive Hx biosensors.

When an aquatic organism dies, degradation of the nucleotides leads to a progressive accumulation of Xa. Quantification of this compound therefore represents a reliable method to evaluate the end of the freshness of fish. Yadav et al. (2012) did studies on Xa to evaluate fish freshness using a n-NiO/PEG/ITO electrode. Similarly, Devi et al. (2012) employed a zinc oxide nanoparticle/chitosan/carboxylated multiwalled carbon nanotube/polyaniline (ZnO-NP/CHIT/c-MWCNT/PANI) electrode to quantify Xa as an indicator of the freshness of labeo fish (*Labeo calbasu*). However, the use of a single compound (Hx or Xa) as a freshness indicator is not always recommended, because many factors can affect nucleotide degradation, such as the type of spoilage bacteria and the mechanical handling of fish.

Table 3 indicates the *K* value, and content of IMP and Hx of varied fish species at different conditions. The initial IMP presented the highest in most species compared with other ATP related compounds. They varied from 5.34 to 26 µmol/g in the selected species. However, the ATP, ADP and AMP in most species remained at low concentration during the entir storage

(Grigorakis et al. 2003; Özogul et al. 2009; Song et al. 2012). The initial Hx remained low in all selected species (ranged from 0 to 3 µmol/g) but increased steadily during spoilage period. But for pike perch (*Sander lucioperca*), Özyurt et al. (2007) observed increase in Hx content during early stage of storage and decrease later stage of storage. The initial *K* value varied significantly from around 0 to 39.7% among species. The high initial *K* value may be owing to excessive struggling during killing.

Flavor Enhancement: Umami

Umami is now generally accepted as the fifth basic taste. It is the term that identifies the taste of substances such as monosodium glutamate (MSG), disodium gluanylate (GMP), and disodium inosinate (IMP). The umami taste was discovered by Ikeda in 1908 (Yamaguchi and Ninomiya, 2000). Since then the concept of umami has been widely accepted in Japan and worldwide. Many papers on umami substances have been published. In 1913, Kodama isolated IMP as a compound to which could be attributed the umami flavor of Kutsuobushi, a molded, dried, Japanese bonito (Noel Rees Jones, 1969). Currently, the “IMP+GMP” or “I+G” has been successfully marketed as a flavor enhancer in oyster sauce, soy sauce, soup stocks, and so on. One of the main I+G producers is the Ajinomoto Company from Japan.

The umami taste has a taste-enhancing synergism between two umami compounds, L-glutamate and IMP. The IMP induces a weak umami taste but can remarkably lower the detection threshold of MSG. Yamaguchi and Ninomiya (2000) proposed an interesting

hypothesis: that the IMP itself may not have the inherent umami taste, but simply strengthens the umami taste of the glutamate generally present in the mouth. Human saliva normally contains a little amount of glutamate (1.5 ppm MSG equivalents). The noticeable umami taste of IMP may actually result from the interaction of IMP with this small concentration of glutamate present in saliva. However, this hypothesis has not been confirmed. The mixing of glutamate and inosinate (IMP in its salt form, usually with sodium) could boost the taste of umami. For instance, kombu dashi (certain kinds of seaweed) alone does not generate a strong umami taste, but the sharp umami taste can be achieved by adding bonito flakes or dried sardines containing inosinate (Kurihara, 2009). Similarly, L-theanine can elicit an umami taste with IMP (Narukawa, Morita, and Hayashi, 2008).

Umami substances were widely distributed in fish and shellfish such as sardine, crab, and lobster. These substances can increase the palatability of fish and shellfish when combined with other substances such as the free amino acids of glycine, alanine, arginine, methionine, valine, and proline, and inorganic ions such as sodium, potassium, chlorine and phosphoric acid. Among the umami substances, IMP, GMP, and AMP are abundant in fish and shellfish. According to Komata (1990), dried sardine and bonito have a very high IMP content (863 and 687 mg/100g, respectively). Chen and Zhang (2007) found that AMP (75.3 mg/100 g), IMP (34.4 mg/100 g) and GMP (2.3 mg/100 g) contributed a strong umami taste to Chinese mitten crab (*Eriocheir sinensis*) meat. Hwang et al. (2000) found that among the taste-active nucleotide compounds,

IMP and GMP were the most prominent in the muscle of puffer fish (*Takifugu rubripes*). Fresh fish generally contains little free IMP and thus very less umami taste. But the umami flavor of fish will increase after being held for several hours as the gradual degradation of ATP begins.

The disappearance of IMP has been correlated with the loss of fresh fish flavor in some species. Meanwhile, the accumulated Hx together with some amino acids and peptides may contribute to a bitter taste in meat (Hernández-Cázares et al., 2011). Therefore, an effort should be made to increase the initial ATP concentration at the time of slaughter, which could result in products with high IMP concentrations at the time of consumption. Handling should also be designed to maximize retention of IMP and minimize the accumulation of Hx.

FACTORS AFFECTING ATP-RELATED COMPOUNDS

The specific pathways and kinetics of degradation of ATP-related compounds are related to the properties of the enzymes associated with each reaction (Figure 1). Numerous factors can directly or indirectly affect the rate of action of the different ATP degradation enzymes.

Species

The ATP and related compounds have been used for quality evaluation of fish or shellfish for over 50 years. However, one of the main reasons for not using the change in ATP catabolites as a universal fish or shellfish quality indicator is that the degradation patterns of ATP catabolites are species-dependent.

Howgate (2006) reviewed the kinetics of degradation of IMP in 45 species of fish during

chilled storage. The results showed that the 45 species have distinct initial concentrations of ATP-related compounds and follow different degradation kinetics, i.e., the reaction order is different. Silver scabbard (*Lepidopus caudatus*) had the highest initial concentrations of IMP (16.6 μmol g^{-1}), whereas for other fish the values ranged between 2.8 and 13.5 μmol g^{-1}. For most fresh fish the reported initial *K* value was below 10%, including such species as bream (*Megalobrama amblycephala*) (Song et al., 2011), grass carp (*Ctenopharyngodon idellus*) (Zhang et al., 2011), bighead carp (*Aristichthys nobilis*) (Hong et al., 2012a) and crucian carp (*Carassius carassius*) (Yao et al., 2011), with *K* values of 5.9, 8.3, 5.3, and 2.4%, respectively. IMP was the main nucleotide present in fish species, whereas AMP was the major component in crustaceans (Mendes et al., 2001). This was proved earlier by Hiltz and Dyer (1970) who found that AMP was the major nucleotide catabolite in the adductor muscle of the scallop (*Placopecten rnagellanicus*); AMP accumulated to a maximum level by the second day post-mortem in ice, while IMP was not detected.

Storage

The degradation of ATP-related compounds increases as the temperature increases above the freezing point of muscle for most fish harvested from temperate waters. Storage of turbot in an ice slurry slowed-down nucleotide degradation (Rodríguez et al., 2006). However, between -0.8 and -5 °C, ATP degradation and glycolysis proceed at a higher speed than at ambient temperature. This is believed to be due to the fact that intracellular water is only partially frozen

between -0.8 and -5°C, which concentrates all the soluble components in the water phase. Meanwhile, organelle membranes may be pierced by ice crystals, resulting in the release of calcium ions from the sarcoplasmatic reticulum. The presence of calcium causes an activation of glycolysis and actomyosin ATPase (Van den Thillart et al., 1990). Therefore, rapid freezing is needed to pass through this range of temperatures as quickly as possible to optimize the quality of frozen fish.

Handling

The death struggle of fish when harvested will significantly accelerate the degradation of ATP-related compounds. Fraser et al. (1965) found that struggling of the fish in a trap and during boating was sufficient to cause a partial depletion of the muscle energy reserve and to boost dephosphorylation and deamination of the nucleotide compounds. One possible solution is to employ early post-harvest icing treatments. This method has successfully been adopted by Badiani et al. (2013) to slow down ATP degradation of cuttlefish (*Sepia officinalis* L.) after they were hauled onboard.

The main synthesis of ATP in post-mortem fish and shellfish was controlled by the amount of glycogen present in the muscle. Glycogen consumption during and after slaughter depends on the fishing technique and killing method employed. Muscle glycogen reserves may be almost depleted even before slaughtering takes place, after which ATP degradation is accelerated, because the muscular work done during capture is often intensive,

Numerous authors have found that immediate spiking of the fish brain (Ikejime in Japanese) or destruction of the spinal cord could slow down the glycogen depletion and ATP degradation. One notable example was published by Mishima et al. (2005) for horse mackerel (*Trachurus trachurus*), who found that the increase in *K* value was the slowest with spinal cord destruction compared with the instant killing (bleeding to death by making a cut at the back of the head), struggling, or temperature shock. Similar results were also reported by Ando et al. (1996), where spinal cord destruction delayed ATP consumption and the process of post-mortem rigor development in yellowtail (*Seriola quinqueradiata*) and red sea bream (*Pagrus major*).

Bleeding fish after harvest also can retard the degradation of ATP and related compounds. Ahimbisibwe et al. (2010) found that the *K* value and the levels of Hx were lower in the muscle tissue of bled fish than in the unbled tissue of amberjack (*Seriola dumerili*) and red sea bream.

Electrical stimulation of muscles causes a rapid depletion of ATP and PCr (Atsushi et al., 1990). Scherer et al. (2005) also found that fish killed using an electric current showed a faster initial rate of ATP degradation and entered into *rigor mortis* earlier. However, a slower ATP depletion was observed in smoked rainbow trout stunned using electricity or bleeding by resecting of the gills compared to the same product obtained by fish slaughtered by simple anoxia (Giuffrida et al., 2007). This discrepancy is perhaps due to species-specific differences.

Irradiation treatments could inhibit nucleotide degradation of sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*) stored in ice (Özogul et al., 2010a; Özogul et al., 2010b).

A high pressure treatment could also slow down nucleotide degradation and decrease the *K* value, possibly due to the suppression of IMP decomposition brought about by inactivation of the dephosphorylases involved in the degradation of ATP and related compounds during high-pressure processing (HPP) (Ginson et al., 2012; Kamalakanth et al., 2011).

Chemical treatments could also affect ATP-related compounds post-mortem in fish and shellfish. Li et al. (2012) reported that tea polyphenols and rosemary extracts could inhibit the degradation of ATP and maintain the quality of fish. Sallam (2007) reported significant reductions in *K* value and Hx concentration in salmon slices treated by dipping them in a 2.5% aqueous solution of sodium acetate, sodium lactate, or sodium citrate during refrigerated storage at 1 °C, compared to the control at the same temperature.

Seasons

Grigorakis et al. (2003) reported that the *K* value of summer sea bream (*Sparus aurata*) was found to be higher initially than that of fish sampled in the winter in the early stages of storage, but lower in the later stages when microbial spoilage occurred. However, Mørkøre et al. (2010) found that the final *K* value in salmon (*Salmo salar* L.) had the lowest value in April (77.9%) and highest value in October (92.9%) after 13 days of chilled storage, while the *K* value was similar in February and August (83%). Hwang et al., (2000) determined that the levels of IMP and AMP were much higher in the muscle of puffer fish (*Takifugu rubripes*) collected from July to January compared with other times of the year. The variation of nucleotides in these fish during different

seasons might be due to different environmental and nutritional conditions, and to a relationship with the spawning period.

CHALLENGE AND FUTURE PERSPECTIVE

From the literature reviewed above, it is apparent that ATP-related compounds play vital roles in the quality changes of post-mortem fish and shellfish. The challenges for future investigations of the role of ATP-related compounds in post-mortem fish and shellfish should focus on optimizing their use in freshness evaluation and quality control of fish and shellfish products. It is suggested that further work should be done as follows:

- The use of ATP metabolites as freshness indexes is a research technique that is not widely used in industry owing to the time and expense involved in these measurements. The future development of this approach to freshness monitoring requires cheap, stable and rapid methods to measure ATP-related compounds.
- Although a variety of analytical methods have been employed to detect ATP-related compounds, it is generally agreed that the HPLC method is the most reliable at this time. But biosensors are one of the most promising directions and further studies should be done on the design of a portable electronic device based on potentiometric measurements for the fast and nondestructive judgment of fish freshness. Biosensors and other rapid methods should be explored further.
- Developing a better understanding of the factors that control the rate of the different

degradation process of ATP-related compounds in each of the commercially important fish species where freshness is a concern.

- Exploring new ways to control the enzymes involved in ATP degradation to both delay the onset of *rigor mortis* and to optimize the amount of IMP present.
- Finding the post-mortem times when IMP accumulates the most for different species in which the umami taste is optimized and adjust fish and shellfish marketing accordingly to provide consumers with the best product for consumption.
- Patterns of spoilage can vary widely even within species. It is most unlikely that the analysis of any single compound would be adequate to cover all aspects. Thus, it is quite important to establish standard freshness indicators based on ATP-related compounds to overcome the variability. It also must be recognized that these freshness indicators will become less important as the fish or shellfish comes closer to microbial spoilage.

CONCLUSION

The catabolism of ATP and related compounds are increasingly attracting research attention, because these factors affect the ultimate eating quality of fish and shellfish. This review has highlighted the progress made in understanding the role of ATP-related compounds in the freshness and flavor of post-mortem fish and shellfish muscle. Considerable effort should be directed towards further optimization of the amount of IMP present in fish and shellfish postmortem.

ACKNOWLEDGEMENT

This work was supported by the earmarked fund for China Agriculture Research System (CARS-46) and the National Natural Science Foundation of China (award no. 31471683). Thanks to China Scholarship Council (CSC) for visiting student program.

References

Alasalvar, C., Taylor, K.D.A., Öksüz, A., Garthwaite, T., Alexis, K. and Grigorakis, K. (2001). Freshness assessment of cultured sea bream (*Sparus aurata*) by chemical, physical and sensory methods. *Food Chem.* **72**:33–40.

Alasalvar, C., Taylor, K. D. A., and Shahidi, F. (2002). Comparative quality assessment of cultured and wild sea bream (*Sparus aurata*) stored in ice. *J. Agric. Food Chem.* **50**: 2039–2045.

Atsushi, C., Masamitsu, H., Masaaki, K., Toshiharu, A., Tatsuya, T., and Shiko, C. (1990). In vivo 31P-NMR analysis of the electric anesthetized loach, *Cobitis biswae*. *Comp. Biochem. Physiol. A: Physiol.* **97**: 385–389.

Aubourg, S. P., Piñeiro, C., Gallardo, J. M., and Barros-Velazquez, J. (2005). Biochemical changes and quality loss during chilled storage of farmed turbot (*Psetta maxima*). *Food Chem.* **90**: 445–452.

Badiani, A., Bonaldo, A., Testi, S., Rotolo, M., Serratore, P., Giulini, G., Pagiluca, G. and Gatta, P. P. (2013). Good handling practices of the catch: The effect of early icing on the freshness quality of cuttlefish (*Sepia officinalis L.*). *Food Control*. **32**: 327–333.

Barat, J. M., Gil, L., García-Breijo, E., Aristoy, M.-C., Toldrá, F., Martínez-Máñez, R., and Soto, J. (2008). Freshness monitoring of sea bream (*Sparus aurata*) with a potentiometric sensor. *Food Chem.* **108**: 681–688.

Burns, G., Ke, P., and Irvine, B. (1985). Objective procedure for fish freshness evaluation based on nuleotide changes using a HPLC system. *Can. Tech. Rep. Fish. Aquat. Sci.* **1373**: 1–39.

Carsol, M., and Mascini, M. (1998). Development of a system with enzyme reactors for the determination of fish freshness. *Talanta*. **47**: 335–342.

Chen, D.-W., and Zhang, M. (2007). Non-volatile taste active compounds in the meat of Chinese mitten crab (*Eriocheir sinensis*). *Food Chem.* **104**: 1200–1205.

Cheng, J., Sun, D.-W., Zeng, X.-A., and Liu, D. (2013). Recent advances in methods and techniques for freshness quality determination and evaluation of fish and fish fillets: A review. *Crit. Rev. Food Sci. Nutr*. (in press). DOI: 10.1080/10408398.2013.769934

Delbarre-Ladrat, C., Chéret, R., Taylor, R., and Verrez-Bagnis, V. (2006). Trends in postmortem aging in fish: understanding of proteolysis and disorganization of the myofibrillar structure. *Crit. Rev. Food Sci. Nutr.***46**: 409–421.

Devi, R., Batra, B., Lata, S., Yadav, S., and Pundir, C. S. (2013). A method for determination of xanthine in meat by amperometric biosensor based on silver nanoparticles/cysteine modified Au electrode. *Process Biochem.* **48**: 242–249.

Devi, R., Thakur, M., and Pundir, C. S. (2011). Construction and application of an amperometric xanthine biosensor based on zinc oxide nanoparticles-polypyrrole composite film. *Biosens. Bioelectron.* **26**: 3420–3426.

Devi, R., Yadav, S., and Pundir, C. S. (2012). Amperometric determination of xanthine in fish meat by zinc oxide nanoparticle/chitosan/multiwalled carbon nanotube/polyaniline composite film bound xanthine oxidase. *The Analyst*. **137**: 754–759.

Ehira, S., and Uchiyama, H. (1974). Freshness-lowering rates of cod and sea bream viewed from changes in bacterial count, total volatile base- and trimethylamine-nitrogen, and ATP related compounds. *B. Jpn. Soc. Sci. Fish.* **40**: 479–487.

Fraser, D. I., Weinstein, H. M., and Dyer, W. J. (1965). Post-mortem glycolytic and associated changes in the muscle of trap- and trawl-caught cod. *J. Fish. Res. Board Can.* **22**: 83–100.

Geldart, S. E., and Brown, P. R. (1998). Analysis of nucleotides by capillary electrophoresis. *J. Chromatogr. A.* **828**: 317–336.

Ghosh (Hazra), S., Sarker, D., and Misra, T. N. (1998). Development of an amperometric enzyme electrode biosensor for fish freshness detection. *Sensor. Actuat. B-Chem.* **53**: 58–62.

Gil, L., Barat, J. M., Garcia-Breijo, E., Ibañez, J., Martínez-Máñez, R., Soto, J., LIobet, E., Brezmes, J., Aristoy, M.-C., and Toldrá, F. (2008). Fish freshness analysis using metallic potentiometric electrodes. *Sensor. Actuat. B-Chem.* **131**: 362–370.

Gill, T. A., Thompson, J. W., Gould, S., and Sherwood, D. (1987). Characterization of quality deterioration in yellowfin tuna. *J. Food Sci.* **52**: 580–583.

Ginson, J., Kamalakanth, C. K., Bindu, J., Venkateswarlu, R., Das, S., Chauhan, O. P., and Gopal, T. K. S. (2012). Changes in K value, microbiological and sensory acceptability of high pressure processed indian white prawn (*Fenneropenaeus indicus*). *Food Bioprocess Tech.* **6**: 1175–1180.

Giuffrida, A, Pennisi, L., Ziino, G., Fortino, L., Valvo, G., Marino, S., and Panebianco, A. (2007). Influence of slaughtering method on some aspects of quality of gilthead seabream and smoked rainbow trout. *Vet. Res. Commun.* **31**: 437–446.

Grigorakis, K., Taylor, K. D., and Alexis, M. (2003). Seasonal patterns of spoilage of ice-stored cultured gilthead sea bream (*Sparus aurata*). *Food Chem.* **81**: 263–268.

Hamada-Sato, N., Usui, K., Kobayashi, T., Imada, C., and Watanabe, E. (2005). Quality assurance of raw fish based on HACCP concept. *Food Control*. **16**: 301–307.

Hernández-Cázares, A. S., Aristoy, M.-C., and Toldrá, F. (2011). Nucleotides and their degradation products during processing of dry-cured ham, measured by HPLC and an enzyme sensor. *Meat Sci.* **87**: 125–129.

Hiltz, D. F., and Dyer, W. J. (1970). Principal acid-soluble nucleotides in adductor muscle of the scallop *Placopecten magellanicus* and their degradation during postmortem storage in ice. *J. Fish. Res. Board Can.* **27**: 83–92.

Hong, H., Luo, Y., Zhu, S., and Shen, H. (2012a). Application of the general stability index method to predict quality deterioration in bighead carp (*Aristichthys nobilis*) heads during storage at different temperatures. *J. Food Eng.* **113**: 554–558.

Hong, H., Luo, Y., Zhu, S., and Shen, H. (2012b). Establishment of quality predictive models for bighead carp (*Aristichthys nobilis*) fillets during storage at different temperatures. *Int. J. Food Sci. Tech.* **47**: 488–494.

Howgate, P. (2006). A review of the kinetics of degradation of inosine monophosphate in some species of fish during chilled storage. *Int. J. Food Sci. Tech.* **41**: 341–353.

Huss, H. H. (1995). Quality and quality changes in fresh fish. *Food and Agriculture Organization of the United Nations. http://www.fao.org/docrep/v7180e/v7180e00.htm*.

Hwang, D.-F., Chen, T.-Y., and Jeng, S.-S. (2000). Seasonal variations of free amino acids and nucleotide-related compounds in the muscle of cultured Taiwanese puffer *Takifugu rubripes*. *Fisheries Sci.* **66**: 1123–1129.

Itoh, D., Koyachi, E., Yokokawa, M., Murata, Y., Murata, M., and Suzuki, H. (2013). Microdevice for on-site fish freshness checking based on K-value measurement. *Anal. Chem.* **85**: 10962–10968.

Jones, N. R. (1969). Meat and fish flavors; significance of ribomononucleotides and their metabolites. *J. Agric. Food. Chem.* **17**: 712–716.

Jones, N. R., Murray, J., Livingston, E. I., and Murray, C. K. (1964). Rapid estimations of hypoxanthine concentrations as indices of the freshness of chill-stored fish. *J. Sci. Food Agri.* **15**: 763–774.

Kamalakanth, C. K., Ginson, J., Bindu, J., Venkateswarlu, R., Das, S., Chauhan, O. P., and Gopal, T. K. S. (2011). Effect of high pressure on K-value, microbial and sensory characteristics of yellowfin tuna (*Thunnus albacares*) chunks in EVOH films during chill storage. *Innov. Food Sci. Emerg. Technol.* **12**: 451–455.

Kaminishi, Y., Matsuno, T., Shindo, J., Miki, H., and Nishimoto, J. (1992). A rapid determination of ATP in fish muscle using luciferase of american firefly Photinus pyrarlis. *B. Jpn. Soc. Sci. Fish.* **58**: 1751–1755.

Karube, I., Matsuoka, H., Suzuki, S., Watanabe, E., and Toyama, K. (1984). Determination of fish freshness with an enzyme sensor system. *J. Agric. Food. Chem.* **32**: 314–319.

Khlyntseva, S. V., Bazel, Y. R., Vishnikin, A. B., and Andruch, V. (2009). Methods for the determination of adenosine triphosphate and other adenine nucleotides. *J. Anal. Chem.* **64**: 657–673.

Komata, Y. (1990). Umami taste of seafoods. *Food Rev. Int.* **6**: 457–487.

Kuda, T., Fujita, M., Goto, H., and Yano, T. (2008). Effects of retort conditions on ATP-related compounds in pouched fish muscle. *LWT - Food Sci. Technol.* **41**: 469–473.

Kurihara, K. (2009). Glutamate: from discovery as a food flavor to role as a basic taste (umami). *Am. J. Clin. Nutr*. **90**: 719S–722S.

Kyrana, V. R., and Lougovois, V. P. (2002). Sensory, chemical and microbiological assessment of farm-raised European sea bass (*Dicentrarchus labrax*) stored in melting ice. *Int. J. Food Sci. Tech.* **37**: 319–328.

Lakshmanan, P. T., Antony, P. D., and Gopakumar, K. (1996). Nucleotide degradation and quality changes in mullet (*Liza corsula*) and pearlspot (*Etroplus suratensis*) in ice and at ambient temperatures. *Food Control*. **7**: 277–283.

Lawal, A. T., and Adeloju, S. B. (2012). Mediated xanthine oxidase potentiometric biosensors for hypoxanthine based on ferrocene carboxylic acid modified electrode. *Food Chem.* **135**: 2982–2987.

Li, T., Li, J., and Hu, W. (2013). Changes in microbiological, physicochemical and muscle proteins of post mortem large yellow croaker (*Pseudosciaena crocea*). *Food Control*. **34**: 514–520.

Li, T., Li, J., Hu, W., Zhang, X., Li, X., and Zhao, J. (2012). Shelf-life extension of crucian carp (*Carassius auratus*) using natural preservatives during chilled storage. *Food Chem.* **135**: 140–145.

Lin, D., and Morrissey, M. T. (1994). Iced storage characteristics of Northern squawfish (*Ptychocheilus oregonensis*). *J. Aquat. Food Prod. Technol.* **3**: 25–43.

Lou, S.-N. (1998). Purine content in grass shrimp during storage as Related to Freshness. *J. Food Sci.* **63**: 442–444.

Luong, J. H. T., Male, K. B., Masson, C., and Nguyen, A. L. (1992). Hypoxanthine ratio determination in fish extract using capillary electrophoresis and immobilized enzymes. *J. Food Sci.* **57**: 77–81.

Marlene R. M., Proctor, J. V., and McLoughlin, M. R. I. A. (1992). The effects of anaesthesia and electrical stunning on chemical changes in the myotomal muscle of Salmo salar post-mortem. Royal Irish Academy, Dublin.

Massa, A. E., Palacios, D. L., Paredi, M. E., and Crupkin, M. (2005). Postmortem changes in quality indices of ice-stored flounder (*Paralichthys Patagonicus*). *J. Food Biochem.* **29**: 570–590.

Mendes, R., Quinta, R., and Nunes, M. L. (2001). Changes in baseline levels of nucleotides during ice storage of fish and crustaceans from the Portuguese coast. *Eur.Food Res. Technol.* **212**: 141–146.

Mishima, T., Nonaka, T., Okamoto, A., Tsuchimoto, M., Ishiya, T., Tachibana, K., and Tsuchimoto, M. (2005). Influence of storage temperatures and killing procedures on post-mortem changes in the muscle of horse mackerel caught near Nagasaki Prefecture, Japan. *Fisheries Sci*. **71**:187–194.

Mohan, C. O., Ravishankar, C. N., Gopal, T. K. S., and Kumar, K. A. (2009). Nucleotide breakdown products of seer fish (*Scomberomorus commerson*) steaks stored in O_2 scavenger packs during chilled storage. *Innov. Food Sci. Emerg. Technol.* **10**: 272–278.

Mora, L., Hernández-Cázares, A. S., Aristoy, M.-C., and Toldrá, F. (2010). Hydrophilic interaction chromatographic determination of adenosine triphosphate and its metabolites. *Food Chem.* **123**: 1282–1288.

Morris, S. and Adamczewska, A.M. (2002). Utilisation of glycogen, ATP and arginine phosphate in exercise and recovery in terrestrial red crabs, Gecarcoidea natalis. *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* **133**:813–825.

Mørkøre, T., Rødbotten, M., Vogt, G., Fjæra, S. O., Kristiansen, I. Ø., and Manseth, E. (2010). Relevance of season and nucleotide catabolism on changes in fillet quality during chilled storage of raw Atlantic salmon (*Salmo salar L.*). *Food Chem.* **119**: 1417–1425.

Nakatani, Y., Fujita, T., Sawa, S., Otani, T., Hori, Y., and Takagahara, I. (1986). Changes in ATP-related compounds of beef and rabbit muscles and a new index of freshness of muscle. *Agric. Biol. Chem.* **50**: 1751–1756.

Nanjyo, Y., and Yao, T. (2002). Rapid measurement of fish freshness indices by an amperometric flow-injection system with a 16-way switching valve and immobilized enzyme reactors. *Anal. Chim. Acta.* **470**: 175–183.

Narukawa, M., Morita K., and Hayashi Y. (2008). L-theanine elicits an umami taste with inosine 5'-monophosphate. *Biosci. Biotechnol. Biochem*. **72**: 3015–3017.

Nguyen, A. L., Luong, J. H., and Masson, C. (1990). Determination of nucleotides in fish tissues using capillary electrophoresis. *Anal. Chem.* **62**: 2490–2493.

Niu, J., and Lee, J. Y. (2000). Bulk-modified amperometric biosensors for hypoxanthine based on sol–gel technique. *Sensor. Actuat. B-Chem.* **62**: 190–198.

Ocaño-Higuera, V.M., Maeda-Martínez, A.N., Marquez-Ríos, E., et al. (2011). Freshness assessment of ray fish stored in ice by biochemical, chemical and physical methods. *Food Chem.* **125**: 49–54.

Ocaño-Higuera, V.M., Marquez-Ríos, E., Canizales-Dávila, M., et al. (2009). Postmortem changes in cazon fish muscle stored on ice. *Food Chem.* **116**: 933–938.

Okuma, H., and Watanabe, E. (2002). Flow system for fish freshness determination based on double multi-enzyme reactor electrodes. *Biosens. Bioelectron.* **17**: 367–372.

Olafsdóttir, G., Martinsdóttir, E., Oehlenschläger, J., Dalgaard, P., Jensen, B., Undeland, I., Mackiee, I.M., Henehanf, G., Nielsenc J., and Nilsen, H. (1997). Methods to evaluate fish freshness in research and industry. *Trends Food Sci. Tech.* **8**: 258–265.

Özogul, F., Özden, Ö., Özoğul, Y., and Erkan, N. (2010a). Nucleotide degradation products of gamma-irradiated sea bream (*Sparus aurata*) stored in ice. *Int. J. Food Sci. Tech.* **45**: 2290–2296.

Özogul, F., Özden, Ö., Özoğul, Y., and Erkan, N. (2010b). The effects of gamma-irradiation on the nucleotide degradation compounds in sea bass (*Dicentrarchus labrax*) stored in ice. *Food Chem.* **122**: 789–794.

Özogul, F., Kamari, N., Küley, E., and Özogul, Y. (2009). The effects of ice storage on inosine monophosphate, inosine, hypoxanthine, and biogenic amine formation in European catfish (*Silurus glanis*) fillets. *Int. J. Food Sci. Technol.* **44**: 1966–1972.

Özogul, F., Özogul, Y., and Kuley, E. (2008). Nucleotide degradation and biogenic amine formation of wild white grouper (*Epinephelus aeneus*) stored in ice and at chill temperature (4 °C). *Food Chem.* **108**: 933–941.

Özogul, F., Taylor, K. D. A., Quantick, P. C., and Özogul, Y. (2000). A rapid HPLC-determination of ATP-related compounds and its application to herring stored under modified atmosphere. *Int. J. Food Sci. Tech.* **35**: 549–554.

Özogul, Y., Özogul, F., and Gökbulut, C. (2006). Quality assessment of wild European eel (*Anguilla anguilla*) stored in ice. *Food Chem.* **95**: 458–465.

Özyurt, G., Özogul, Y., Özyurt, C.E., et al. (2007). Determination of the quality parameters of pike perch *Sander lucioperca* caught by gillnet, longline and harpoon in Turkey. *Fish Sci*. **73**: 412–420.

Park, I.-S., and Kim, N. (1999). Simultaneous determination of hypoxanthine, inosine and inosine 5′-monophosphate with serially connected three enzyme reactors. *Anal. Chim. Acta.* **394**: 201–210.

Roberts, B., Morris, B. A., and Clifford, M. N. (1991). Comparison of radioimmunoassay and spectrophotometric analysis for the quantitation of hypoxanthine in fish muscle. *Food Chem.* **42**: 1–17.

Rodríguez, Ó., Barros-Velázquez, J., Piñeiro, C., Gallardo, J. M., and Aubourg, S. P. (2006). Effects of storage in slurry ice on the microbial, chemical and sensory quality and on the shelf life of farmed turbot (*Psetta maxima*). *Food Chem.* **95**: 270–278.

Ryder, J. M. (1985). Determination of adenosine triphosphate and its breakdown products in fish muscle by high-performance liquid chromatography. *J. Agric. Food. Chem.* **33**: 678–680.

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

Sallam, K. I. (2007). Chemical, sensory and shelf life evaluation of sliced salmon treated with salts of organic acids. *Food Chem.* **101**: 592–600.

Scheffler, T. L., Park, S., and Gerrard, D. E. (2011). Lessons to learn about postmortem metabolism using the AMPKγ3(R200Q) mutation in the pig. *Meat Sci.* **89**: 244–250.

Scherer, R., Augusti, P. R., Steffens, C., Bochi, V. C., Heckttheuer, L. H., Lazzari, R., Radünz-neto J., Pomblum, S.C.G., and Emanuelli, T. (2006). Effect of slaughter method on postmortem changes of grass carp (*Ctenopharyngodon idella*) stored in ice. *J. Food Sci.* **70**: C348–C353.

Shahidi, F., Chong, X., and Dunajski, E. (1994). Freshness quality of harp seal (*Phoca groenlandica*) meat. *J. Agric. Food. Chem.* **42**: 868–872.

Shiba, T., Shiraki, N., Furushita, M., and Maeda, T. (2012). Free amino acid and ATP-related compounds in sterile tiger puffer fish (*Takifugu rubripes*) fillets stored at 4C. *J. Food Process. Pres.* **38**: 791–797.

Soga, T., and Imaizumi, M. (2001). Capillary electrophoresis method for the analysis of inorganic anions, organic acids, amino acids, nucleotides, carbohydrates and other anionic compounds. *Electrophoresis*. **22**: 3418–3425.

Soga, T., Ishikawa, T., Igarashi, S., Sugawara, K., Kakazu, Y., and Tomita, M. (2007). Analysis of nucleotides by pressure-assisted capillary electrophoresis-mass spectrometry using silanol mask technique. *J. Chromatogr. A.* **1159**: 125–133.

Song, Y., Liu, L., Shen, H., You, J., and Luo, Y. (2011). Effect of sodium alginate-based edible coating containing different anti-oxidants on quality and shelf life of refrigerated bream (*Megalobrama amblycephala*). *Food Control*. **22**: 608–615.

Song, Y., Luo, Y., You, J., Shen, H., and Hu, S. (2012). Biochemical, sensory and microbiological attributes of bream (*Megalobrama amblycephala*) during partial freezing and chilled storage. *J. Sci. Food Agri.* **92**: 197–202.

Spinelli, J., Eklund, M., and Miyauchi, D. (1964). Measurement of hypoxanthine in fish as a method of assessing freshness. *J. Food Sci.* **29**: 710–714.

Spinelli, J., and Kemp, B. (1966). Nucleotide measurement, rapid measurement of inosine monophosphate and total adenosine nucleotides in fish tissue. *J. Agric. Food. Chem.* **14**: 176–178.

Tanaka, S., Kuroda, A., Kato, J., Ikeda, T., Takiguchi, N., and Ohtake, H. (2001). A sensitive method for detecting AMP by utilizing polyphosphate-dependent ATP regeneration and bioluminescence reactions. *Biochem. Eng. J.* **9**: 193–197.

Tarr, H. L. A. (1966). Post-mortem changes in glycogen, nucleotides, sugar phosphates, and sugars in fish muscles-a review. *J. Food Sci.* **31**: 846–854.

Thandavan, K., Gandhi, S., Sethuraman, S., Rayappan, J. B. B., and Krishnan, U. M. (2013). Development of electrochemical biosensor with nano-interface for xanthine sensing-a novel approach for fish freshness estimation. *Food Chem.* **139**: 963–969.

Tomlinson, N., and Geiger, S. (1962). Glycogen concentration and post mortem loss of adenosine triphosphate in fish and mammalian skeletal muscle. A review. *J. Fish. Res. Board Can.* **19**: 997–1003.

Tseng, H. C., Dadoo, R., and Zare, R. N. (1994). Selective determination of adenine-containing compounds by capillary electrophoresis with laser-induced fluorescence detection. *Anal. Biochem.* **222**: 55–58.

Van den Thillart, G., van Waarde, A., Muller, H. J., Erkelens, C., and Lugtenburg, J. (1990). Determination of high-energy phosphate compounds in fish muscle: 31P-NMR

spectroscopy and enzymatic methods. *Comp. Biochem. Physiol. B: Biochem. Mol. Biol.* **95**: 789–795.

Venugopal, V. (2002). Biosensors in fish production and quality control. *Biosens. Bioelectron.* **17**: 147–157.

Volpe, G., and Mascini, M. (1996). Enzyme sensors for determination of fish freshness. *Talanta*. **43**: 283–289.

Watanabe, E., Tamada, Y., and Hamada-Sato, N. (2005). Development of quality evaluation sensor for fish freshness control based on K_I value. *Biosens. Bioelectron.* **21**: 534–538.

Watanabe, E., Tokimatsu, S., Toyama, K., Karube, I., Matsuoka, H., and Suzuki, S. (1984). Simultaneous determination of hypoxanthine, inosine, inosine-5′-phosphate and adenosine-5′-phosphate with a multielectrode enzyme sensor. *Anal. Chim. Acta.* **164**: 139–146.

Yadav, S. K., Singh, J., Agrawal, V. V., and Malhotra, B. D. (2012). Nanostructured nickel oxide film for application to fish freshness biosensor. *Appl. Phys. Lett.* **101**: 1-3.

Yamaguchi, S., and Ninomiya, K. (2000). Umami and food palatability. *J. Nutr.* **130**: 921S–926S.

Yao, L., Luo, Y., Sun, Y., and Shen, H. (2011). Establishment of kinetic models based on electrical conductivity and freshness indictors for the forecasting of crucian carp (*Carassius carassius*) freshness. *J. Food Eng.* **107**: 147–151.

Zhang, L., Li, X., Lu, W., Shen, H., and Luo, Y. (2011). Quality predictive models of grass carp (*Ctenopharyngodon idellus*) at different temperatures during storage. *Food Control*. **22**: 1197–1202.

Zur Nedden, S., Eason, R., Doney, A. S., and Frenguelli, B. G. (2009). An ion-pair reversed-phase HPLC method for determination of fresh tissue adenine nucleotides avoiding freeze-thaw degradation of ATP. *Anal. Biochem.* **388**: 108–114.

Table 1 Chromatographic methods for the determination of ATP-related compounds in post-mortem fish

Method	Detection technique	Analytes	Sample pretreatment	Mobile phase	References
Ion-exchange chromatography	Optical density	Hx, Ino	4% perchloric acid	0.01N ammonium chloride	(Saito & Arai, 1959)
	UV detector 250 nm	ATP, ADP, AMP, IMP, NAD, GMP	3% perchloric acid	Distilled water, then 1N H_2SO_4, then 6N H_2SO_4	(Spinelli & Kemp, 1966)
Ion-pairing HPLC	UV detector 254 nm	ATP, ADP, AMP, IMP, GTP, CTP, GDP, Adenosine, inosine, GMP, Xa, HX	5% perchloric acid	A: 39mM K_2HPO_4 and 26mM KH_2PO_4, adjusted to pH 6 with orthophosphoric acid and 4mM TBAHS B: 39mM K_2HPO_4 and 26mM KH_2PO_4, adjusted to pH 6 with orthophosphoric acid and 25% methanol	(Zur Nedden et al., 2009)
Revere-phase HPLC	UV detector 254 nm	ATP, ADP, AMP, IMP, Ino, Hx	10% perchloric acid	0.05M sodium phosphate buffer (pH 6.8)	(Zhang et al., 2011)
	UV detector 250 nm	ATP, ADP, AMP, IMP, Ino, Hx	5% perchloric acid	0.05M sodium di-hydrogen phosphate (pH 4.0)	(Kuda et al., 2008)
	Diode array detector	ATP, ADP, AMP, IMP, Ino, Hx	0.6 M perchloric acid	A: 0.04M KH_2PO_4 and 0.06M K_2HPO_4 B: acetonitrile	(Özogul et al., 2000)
	UV detector 260 nm	ATP, ADP, AMP, IMP, Ino, Hx	5% perchloric acid	0.1M phosphate-triethylamine buffer (pH 6.8) and 1.35% acetonitrile	(Shiba et al., 2012)
Capillary electrophoresis	UV detector 250 nm	Hx, Ino, IMP	10% trichloroacetic acid	0.1M CAPS buffer, pH 11, 30kV, 50μA	(Luong et al., 1992)

TBAHS: tetrabtylammonium hydrogen sulfate, CAPS: 3-[cyclohexylaminol-1-propanesulfonic acid]

Table 2 Indicators of freshness using ATP-related compounds for fish and shellfish

Indicator	Calculation	Species	Reference for first report	Remarks
K value	[(Ino+Hx)/(ATP+ADP+AMP+ IMP+Ino+Hx)]×100	Various sea fish	(Saito & Arai, 1959)	One of the most effective indicators of fish freshness
K_0 value	[(Ino+Hx)/(AMP +ADP+ IMP+Ino+Hx+X)]×100	Beef and rabbit muscles	(Nakatani et al., 1986)	Also applicable to fish and shellfish
K_I, K_i or K_1 value	[(Ino+Hx)/(IMP+Ino+Hx)]×100	Sea bass, mackerel, saurel, and yellowfish	(Karube et al., 1984)	Highly correlated with K value
H value	[Hx/(IMP+Ino+Hx)]×100	Atlantic cod, trout, salmon	(Luong et al., 1992)	Indicator of bitter "off-taste"
P value	[(Ino+Hx)/(AMP+IMP+Ino+Hx)]×100	Queen crab, mackerel, cod	(Burns et al., 1985)	Serve as an indicator of spoilage during the early stages of chilled storage
G value	[(Ino+Hx)/(AMP+IMP+Ino)]×100	Queen crab, mackerel, cod	(Burns et al., 1985)	Useful with lean fish
Fr or IMP ratio	[IMP/(IMP+Ino+Hx)]×100	Tuna	(Gill et al., 1987)	Appropriate for species where IMP degrades gradually
Kp value	Hx/Adenine	Grass shrimp (*Penaeus monodon*)	(Lou, 1998)	Applicable during 5°C and 22°C storage
Hx	Hypoxanthine	-	(Jones et al., 1964; Spinelli et	-

			al., 1964)	
Xa	Xanthine	*Labeo rohita*	(Devi et al., 2012)	Can be detected by electrochemical biosensor with nano-interface

Table 3 K value, and content of IMP and Hx of varied fish species at different conditions

Species	Temperature/time	IMP (μmol/g)	Hx (μmol/g)	K value (%)	Reference
Chub mackerel (*Scomber japonicus*)	Raw/day 0	10.70	0.07	8.9	(Kuda et al. 2008)
Yellowfin tuna (*Thunnes albacares*)	Raw/day 0	10.02	0.62	13.7	(Kuda et al. 2008)
Pink salmon (*Oncorhynchus gorbuscha*)	Raw/day0	5.34	0.91	39.7	(Kuda et al. 2008)
cazon fish (*Mustelus lunulatus*)	Ice/ day 0	6.35	0.12	1.1	(Ocaño-Higuera et al. 2009)
Ray fish (*Dasyatis brevis*)	Ice/0day	6.15	0.10	4.7	(Ocaño-Higuera et al. 2011)
Seer fish (*Scomberomorus commerson*)	0-2°C/day 0	8.8	N.D.	3.54	(Mohan et al. 2009)
European catfish (*Silurus glanis)*	Ice/day 0	12.6	<3.00	50	(Özogul et al. 2009)
Gilthead sea bream (*Sparus aurata*)	Ice/day 0, winter	8.21	0.06	0.90	(Grigorakis et al. 2003)
Gilthead sea bream	Ice/day 0, summer	12.05	0.02	0.16	(Grigorakis et al. 2003)
Sea bass (*Dicentrarchus labrax*)	Ice/day 0	11.57	1.27	19.5	(Özogul et al. 2010)
Pearlspot（*Etroplus suratensis*）	Ice/day 0	6.36	0.16	3-4	(Lakshmanan et al. 1996)
Mullet (*Liza corsula)*	Ice/day 0	8.47	0.175	3-4	(Lakshmanan et al. 1996)
Wild white grouper (*Epinephelus aeneus*)	4°C/day 0	~26	0.66	4	(Özogul et al. 2008)
Cultures sea bream (*Sparus aurata*)	Ice/day 0	~8	N.D.	~0	(Alasalvar et al. 2001)

IMP, inosine 5′-monophosphate; Hx, hypoxanthine

Figure Legends

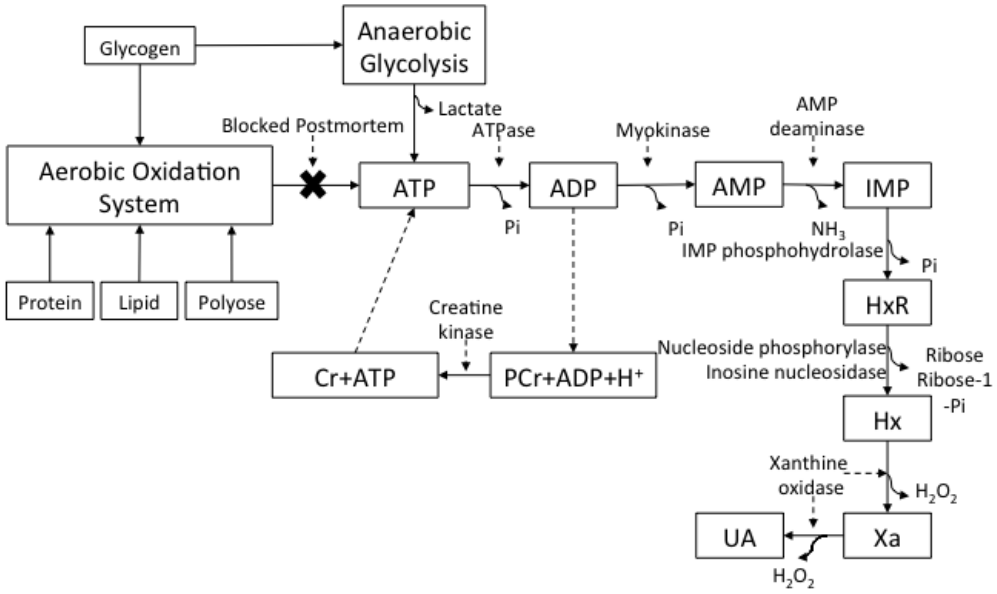

Figure 1. Post-mortem ATP metabolism in fish muscle (modified from Huss (1995)).

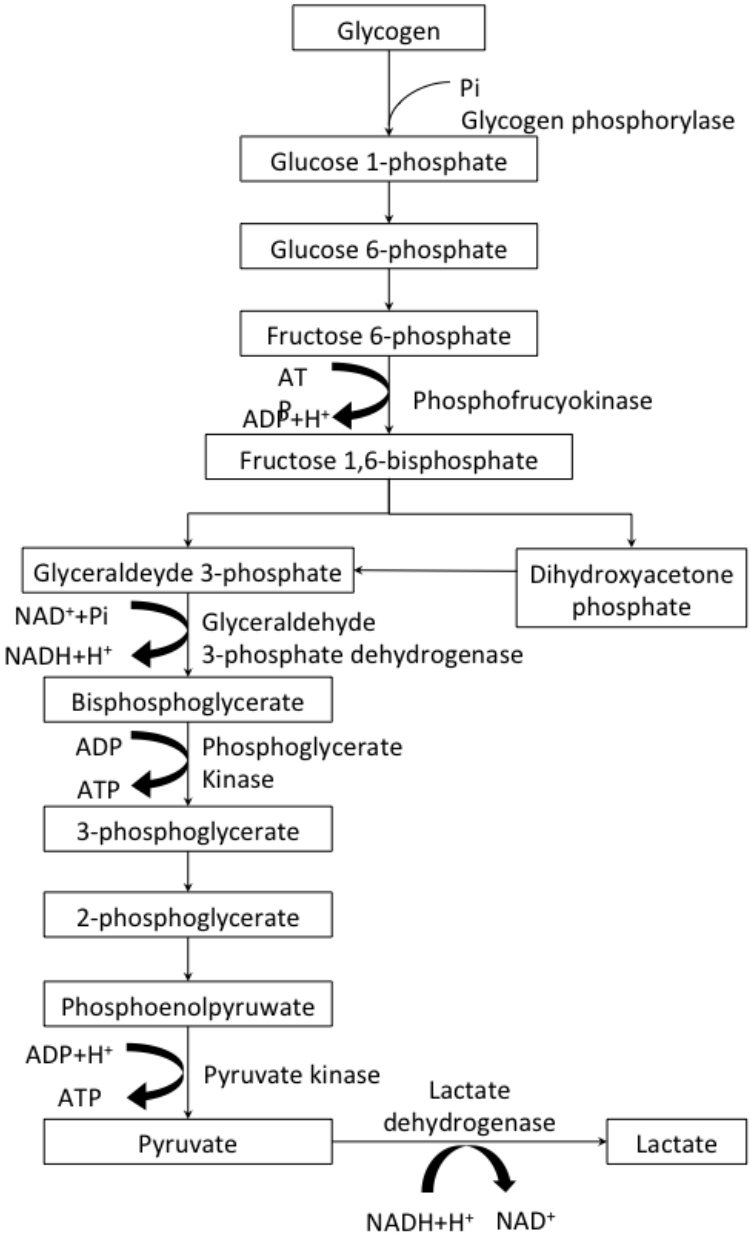

Figure 2. The glycolytic pathway in post-mortem fish muscle (Scheffler et al., 2011).