VTT PUBLICATIONS 297

Adenosine triphosphate breakdown products as a freshness indicator of some fish species and fish products

Tapani Hattula

VTT Biotechnology and Food Research

University of Helsinki,
Department of Biochemistry

ACADEMIC DISSERTATION

To be presented, with the permission of the Biochemistry Department of the Faculty of Mathematics and Natural Science of the University of Helsinki, for public criticism in the lecture hall 1041 of the Department of Biochemistry, Viikinkaari 5, on March 14th, 1997, at 12 o'clock noon.

TECHNICAL RESEARCH CENTRE OF FINLAND
ESPOO 1997

ISBN 951–38–4955–4 (soft back ed.)
ISSN 1235–0621 (soft back ed.)

ISBN 951–38–5063–3 (URL: http://www.inf.vtt.fi/pdf/)
ISSN 1455–0849 (URL: http://www.inf.vtt.fi/pdf/)

JULKAISIJA – UTGIVARE – PUBLISHER

Valtion teknillinen tutkimuskeskus (VTT), Vuorimiehentie 5, PL 2000, 02044 VTT
puh. vaihde (09) 4561, faksi (09)456 4374

Statens tekniska forskningscentral (VTT), Bergsmansvägen 5, PB 2000, 02044 VTT
tel. växel (09) 4561, fax (09) 456 4374

Technical Research Centre of Finland (VTT), Vuorimiehentie 5, P.O.Box 2000, FIN–02044 VTT, Finland
phone internat. + 358 9 4561, fax + 358 9 456 4374

VTT Bio- ja elintarviketekniikka, Elintarviketekniikka, Biologinkuja 1, PL 1500, 02044 VTT
puh. vaihde (09) 4561, faksi (09) 455 2103

VTT Bio- och livsmedelsteknik, Livsmedelsteknik, Biologgränden 1, PB 1500, 02044 VTT
tel. växel (09) 4561, fax (09) 455 2103

VTT Biotechnology and Food Research, Food Technology,
Biologinkuja 1, P.O.Box 1500, FIN-02044 VTT, Finland
phone internat. + 358 9 4561, fax + 358 9 455 2103

Technical editing Leena Ukskoski

VTT OFFSETPAINO, ESPOO 1997

Hattula, Tapani. Adenosine triphosphate breakdown products as a freshness indicator of some fish species and fish products. Espoo 1997, Technical Research Centre of Finland, VTT Publications 297. 48 p. + app. 31 p.

UDC 664:579.67:597.2/.5
Keywords adenosine phosphates, chemical indicators, freshness, fishes, fish products, herrings, trout, chromatography, methods, laboratory tests, food industry, quality

Abstract

The breakdown products of adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), inosine monophosphate (IMP), inosine (Ino) and hypoxanthine (Hx) of some fish species and heated fish products were determined by high-performance liquid chromatography (HPLC) and rapid paper strip method.

The *K* value, calculated from ATP degradation products, the percent of the sum of Ino and Hx divided by the sum of ATP, ADP, AMP, IMP, Ino and Hx increased almost linearly in the muscle of all Finnish fish species studied, when stored on ice. There were some variations of *K* value among fish species and also between individuals of the same fish species, while *K*-value did not depend on the location of whitefish fillets.

The *K* value of cold smoked rainbow trout increased similarly to that of unheated fish. In hot smoked fish (whitefish, mackerel and rainbow trout) the *K*-value increased during heating but not at all after subsequent storage in a chill room. The *sous vide* (final internal temperature 73°C) treated whitefish behaved like the hot smoked fish species.

The *K* value of rainbow trout increased more slowly during heating than the *K* value of other fish species (Baltic herring, whitefish and mackerel) and in the case of the hot smoked rainbow trout the *K* value could be an indicator of the freshness of the raw material used. The low *K* value could possibly be used as evidence of really fresh fish.

The *K* value was used as a criterion when comparing the effects of catching method on the quality of Baltic herring. The *K* values of gillnet-caught fish were greater than those of fish caught by trapnet or by trawling. The duration of trawling time (2 h and 5 h) did not effect the *K* value when the water temperature was 8–16 °C.

An intercalibration study was performed with 23 municipal laboratories, which used the *K*-value, determined by means of the HPLC method or rapid paper strip method, as a criterion for fresh, frozen, and frozen fish stored on ice for 7 days before freezing. The paper strip method correlated quite well with the sensory quality of fish while the relationship between *K*-value determined by HPLC and by the paper strip method was not very good.

The determination of ATP breakdown products and the calculation of *K*-value are relevant chemical methods for evaluation of the freshness of fish. However, there is a certain variation between individual fishes and between fish species.

Preface

This study was carried out at VTT Biotechnology and Food Research in Espoo during 1991 - 1996 (in 1991 and 1992, VTT/Food Research Laboratory).

I express my sincere gratitude to my initial supervisors, Docent Martti Kiesvaara and Professor Yrjö Mälkki. I am also very grateful to my present supervisor, Dr. Raija Ahvenainen, who encouraged me patiently during later studies and preparing publications.

I am especially grateful to my technicians Ms Eeva Manninen and Ms Maritta Härkönen, who performed the *K* value analysis conscientiously and bravely obtained samples on the stormy waters of the Baltic sea. I would also like to thank Mr Veli Heinonen for providing fresh whitefish samples from the lake Päijänne.

I thank Ms Irma Salovaara for helping to build up the literature review.

I would like to thank Mr Markku Aro from the Ministry of Agriculture and Forestry, Professor Kaisa Poutanen, Professor Juha Ahvenainen and Mr Antti Mustranta for support of projects joined with my studies.

My two reviewers, Dr. Ian I. Mackie and Dr. Pertti Varo, gave me constructive comments. For this I am very grateful.

The cooperation of Mr Jouko Poutanen, Mr Orian Bundestam and Mr Rauno Kostiainen from the Federation of Fisheries Association, Ms Marja Kallio and Dr. Petri Suuronen from the Finnish Game and Fisheries Research Institute, made it possible for me to obtain really fresh fish during my studies with Baltic herring.

I thank my Professor of Biochemistry, Carl G. Gahmberg and Dr. Anna-Maija Sjöberg for practical instruction concerning the dissertation procedure.

Finally, I would like to thank all the staff of the VTT Biotechnology and Food Research and my family for a positive attitude to my studies.

Espoo, November 1996
Tapani Hattula

List of original publications

This thesis is based mainly on the following original articles, which are referred as Publications I - V.

I Hattula, T. & Kiesvaara, M. 1992. Patterns of adenosine triphosphate catabolism as freshness indicators in fish species from the Baltic Sea. J. Sci. Food Agric., Vol. 58, No. 4, pp. 485 - 488.

II Hattula, T., Kiesvaara, M. & Moran, M. 1993. Freshness evaluation in European whitefish (*Coregonus wartmanni*) during chill storage. J. Food Sci., Vol. 58, No. 6, pp. 1212 - 1215, and 1236.

III Hattula, T., Luoma, T., Kostiainen, R., Poutanen, J., Kallio, M. & Suuronen, P. 1995. Effects of catching method on different quality parameters of Baltic herring (*Clupea harengus* L). Fish. Res., Vol. 23, pp. 209 - 221.

IV Hattula, T. & Kiesvaara, M. 1996. Breakdown products of adenosine triphosphate in heated fishery products as an indicator of raw material freshness and of storage quality. Lebensm.-Wiss. Technol., Vol. 29, pp. 135 - 139.

V Hattula, T. & Wallin, H. 1996. Rapid method based on ATP catabolites for evaluating the freshness of Baltic Herring: Inter-laboratory study. J. AOAC Int., Vol. 79, No. 3, pp. 703 - 706.

Contents

Abbreviations

ATP	adenosine triphosphate
ADP	adenosine diphosphate
AMP	adenosine 5'-monophosphate
DNA	deoxyribonucleic acid
IMP	inosine 5'-monophosphate or inosinic acid
Ino	inosine
HPLC	high-performance liquid chromatography
Hx	hypoxanthine
NT	5'-nucleotidase
NP	nucleoside phosphorylase
RNA	ribonucleic acid
TMA-N	trimethyl amine nitrogen
TVB-N	total volatile basic nitrogen
U	uric acid
XO	xanthine oxidase

1. Introduction

There is an old Eastern-Finnish proverb "The fish and unpleasant quests begin to smell after three days". The saying was really true as far as fish were concerned during certain seasons when fish were stored at ambient temperature and when refrigerators were not commonly available. Fish, unheated or without refrigeration, spoils quite quickly. The really unpleasant smell of fish is derived at its peak when hydrogen sulphide, short-chain fatty acids and amines are evolved by means of the fish enzymes and spoiling microorganisms (Wong *et al.* 1967). After fish has been killed, it has typical flavour. After several days' storage on ice the typical flavours decrease (Whittle *et al.* 1990). If bacterial growth is inhibited, for example using carbon dioxide in modified atmosphere packaging, the flavourless phase of fish is lengthened as compared with the storage of fish without microbicides (Parkin & Brown, 1982).

Sensory changes after the death of fish have been reviewed by Nielsen (1995). The first sensory changes of fish are concerned with appearance and texture. The most important change of texture is caused by *rigor mortis*. Immediately after death the muscle is relaxed. When the muscle becomes stiff the whole body becomes inflexible and the fish is in *rigor mortis*. The onset and duration of *rigor mortis* are different depending on the species and the temperature of the fish (Nielsen 1995). The time from death to the onset of *rigor mortis* varies from 0.5 h (stressed cod) to 60 h (carp at +10°C). The time from death to the end of *rigor mortis* varies from 1 h to 4 days (Nielsen 1995). Development of *rigor mortis* has been linked to depletion of ATP (Korhonen *et al.* 1990).

There are four phases which describe the eating quality of fish (Fig. 1). In the first phase the characteristic taste of the species is developed. The decrease of quality in the second phase is caused by autolysis and in the third phase by bacteria. In the fourth phase the fish is inedible.

Spencer & Baines (1964) proposed that the rate of spoilage (u) was dependent on temperature (T) according to the form

$$u = V(1 + cT) \qquad [1]$$

where V is the spoilage rate at 0°C and c is a constant. Values of c between 0.25 and 0.36 were obtained for different spoilage tests (sensory, chemical and bacteriological) used, these being similar to earlier published results.

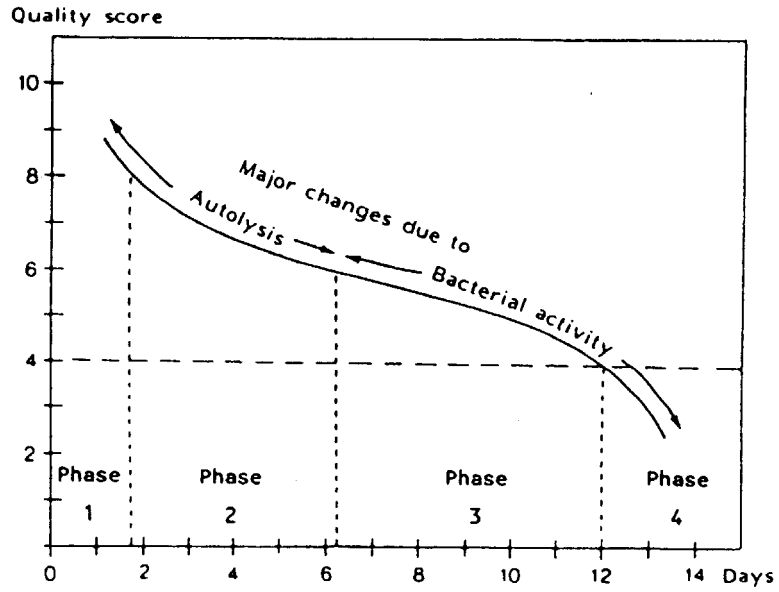

Figure 1. Changes in the eating quality of iced (0°C) cod (Huss 1976).

Besides the sensory analysis, the freshness of fish has been evaluated by chemical methods (Connell 1980), and on the basis of the dielectric properties of fish skin and muscle (Jason and Richards1975). Connell (1980) pointed out that sensory assessment is the crucial and final method of evaluating fish freshness. Botta (1994) reviewed methods concerning the evaluation of the freshness quality of seafood and realized that it is incorrect to assume that there is no objectivity in sensory evaluation. Sensory evaluation has been, and is still the most usual way to evaluate fish quality among fishermen, people working in fish plants, among fish wholesalers and vendors.

In order to attain a better understanding of fish spoiling, biochemical reactions have been studied. The old indicators of fish freshness have been trimethylamine (TMA) and total count of viable bacteria. It is, however, found that they are indicators for advanced spoiling but not a criteria for the loss of freshness as are nucleotides (Ashie *et al.* 1996). In this sense, this work was undertaken to study the changes in nucleotides in Finnish fish.

Nucleotides are the second most abundantly occurring nitrogen-containing extracts of fish after free amino acids (Borresen 1995b). ATP-related compounds of fish have been analyzed as freshness indicators during the many years since Saito *et al.* published their discoveries in 1959 "A new method for estimating the freshness of fish".

2. Literature review

2.1. Biochemistry of fish muscle, and enzymes degrading ATP and its related compounds

The principal constituents of fish muscle are water (66 - 81 %), proteins (16 - 21 %), lipids (0.2 - 25 %), and ash (1.2 - 1.5 %) (Borresen 1995b). The myofibrillar proteins, actin and myosin (about 2/3 of total proteins) play an essential role when a fish swims. The water-soluble proteins (20 - 35 %) are mostly enzymes (Borresen 1995b; Mackie 1993). The proportion of water-soluble proteins in the total protein content does not change during chill storage (Laird *et al.* 1980). The identification of fish species has been based on this fraction because the water-soluble proteins differ depending on the fish species (Mackie 1980).

Fish muscle is comprised of dark and white muscle or ordinary muscle. The proportion of dark to white muscle varies between species, increasing with swimming activity (Love 1974). There is much less dark muscle in gadoid fish, which often rest on the sea bottom, than in the pelagic fish which swim continuously throughout their lives.

Muscle contraction starts when a nervous impulse sets off a release of Ca^{++} cations from the sarcoplasmic reticulum to the myofibrils. The enzyme ATPase is activated by Ca^{++} cations. The ATPase splits the ATP found between the actin and myosin filaments, causing a release of energy. This energy is used as contractile energy making the actin filaments slide in between the myosin filaments in a telescopic fashion, thereby contracting the muscle fibre (Borresen 1995a).

Kamal *et al.* (1991) found that the ATPase activity of sardine muscles decreased during ice storage. Nambudiri & Gopakumar (1992) found that the ATPase activity of several fish species decreased during frozen storage (-20^{o}C).

The enzymes degrading ATP and its related compounds in bonefish are presented in Fig. 2.

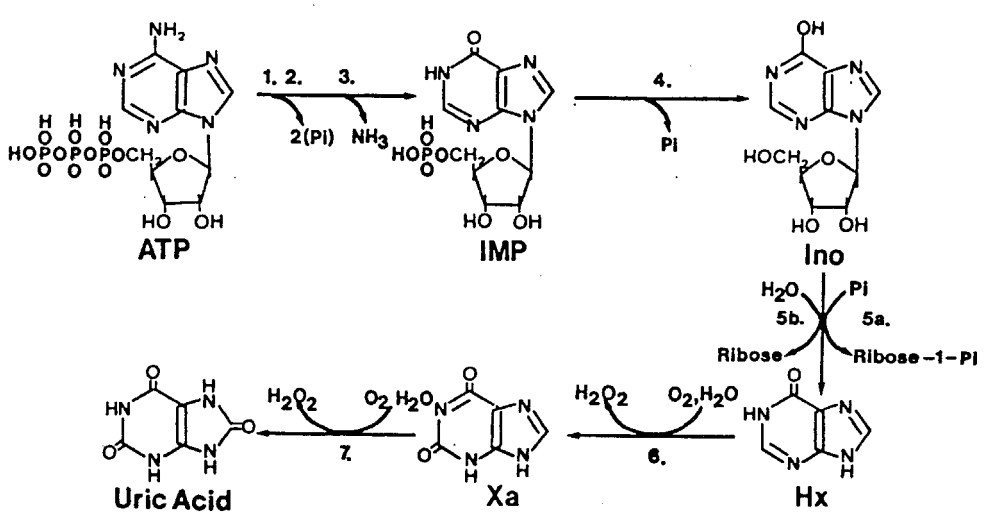

Figure 2. Post-mortem ATP degradation in bonefish. Enzymes include: 1. ATPase, 2. myokinase, 3. AMP deaminohydrolase (or AMP deaminase), 4. IMP phosphohydrolase (5'-nucleotidase), 5a. nucleoside phosphorylase, 5b. inosine nucleosidase, 6,7. xanthine oxidase (Gill 1992).

The ATP degrading enzyme, ATPase is in the myofibrillar fraction of fish muscle (Borresen 1995a). Dingle & Hines (1967) made an aqueous extract of adenosine 5'-monophosphate aminohydrolase from cod (*Gadus morhua*) muscle. Thus, the complex of this enzyme with actomyosin does not seem to exist in cod as is the case in some mammalian muscle. In some cases AMP aminohydrolase can be dissociated from myosin by heat treatment. Raffin & Leray (1980) performed a comparative study on AMP deaminase between different fish species (*Anguilla anguilla*, *Perca fluviatilis*, *Cyprinus carpio*, *Esox lucius*, and *Scyliurhinus* spp.) and between different fish tissues. The highest activity of the enzyme was found in the muscle of northern pike. A low activity is generally found in the blood of fish, but a relatively high activity is found in the blood of a selachian fish (*Scyliurhinus* spp.). There is quite active AMP deaminase in the gill of the fish. Makarewicz & Zydowo (1962) examined the excretion of nitrogen in different tissues and found that the adenosine nucleotides played an important part in the nitrogen metabolism of the fish gill. Makarewicz (1969) purified AMP aminohydrolase from elasmobranch fish (*Raja clavata* L). In this case the enzyme was extracted with dilute phosphate buffer and they proved that AMP aminohydrolase was not firmly bound to actomyosin.

Marseno *et al*. (1992) studied the distribution of 5'-nucleotidases in muscle homogenates of some vertebrates and invertebrates. The highest activity was found in black rock fish (*Sebastes inermis*). Marseno *et al.* (1993a, 1993b) isolated different subcellular fractions from black rock fish and

found that the 5'-nucleotidase activity was in the nuclear, microsomal and cytosolic fractions. IMP degrading enzymes are mostly within the water-soluble fraction (Jessen 1995). Nedachi & Hirota (1992) isolated the 5'-nucleotidase, which splits phosphate from IMP, from the water-soluble fraction of snapper (*Pagrus auratus*). Marseno *et al.* (1993b) have isolated 5'-nucleotidase both from the membranes and cytosol of black rockfish (*Sebastes inermis*). 5'-Nucleotidase has also isolated from carp (Tomioka & Endo 1984) and from cod muscle (Yamamoto *et al.* 1986).

Tarr (1955) isolated riboside hydrolase from a 0.6 M NaCl extract of lingcod (*Ophiodon elongatus*) and rock cod (*Sebastodes* sp.) which indicates that riboside hydrolase is present in the myofibrillar fraction of fish. The accumulation of Ino and Hx appears to be related to both autolytic and microbial action (Surette, 1987). Boyle *et al.* (1991) found that Hx increased more slowly in a CO_2 atmosphere than in a normal atmosphere. It is known that certain bacteria do not grow in a CO_2 atmosphere and in this case do not produce Hx. Dipping of fillets in potassium sorbate solution also inhibited the increase of Hx, which indicates that potassium sorbate prevents the growth of bacteria and consequently the formation of Hx.

Yokoyama *et al.* (1994) examined post-mortem changes of ATP and its related compounds in spear squid (*Dorotheus bleekeri*). They found that degradation of AMP proceeded through two pathways, the IMP and adenosine pathways, in all three muscles. The adenosine pathway is more active than the IMP pathway. Mollusks rarely have AMP deaminase, so that their major pathway is

ATP -> ADP -> AMP -> adenosine -> Ino -> Hx

showing that the catabolism of ATP is reminiscent of squid (Konosu & Yamaguchi 1982).

The IMP pathway prevails in elasmobranchs, because there is plenty of IMP in the muscle of shark (Konosu & Yamaguchi 1982).

2.2. Traditional chemical assessment of fish freshness

The traditional chemical means for evaluation of fish freshness have been the determination of the concentrations of total volatile basic nitrogen (TVB-N) and trimethylamine nitrogen (Pearson 1970). Hebard *et al.* (1982) reviewed the occurrence and the significance of TMAO and its derivatives

in fish. In certain fish species, for example in Baltic herring (*Clupea harengus membras* L), TVB-N is a better criterion than trimethylamine nitrogen (TMA-N), because there is a very low concentration of the precursor of TMA, namely trimethylamine oxide (Nikkilä 1951). The determination of TVB-N was better than that of TMA when analysing the freshness of vendace (*Coregonus albula* L), whitefish (*Coregonus lavaretus* L), perch (*Perca fluviatilis* L) and rainbow trout (*Salmo gairdneri R)* (Hattula & Kiesvaara 1982).

Ashie *et al.* (1996) reviewed the microbes causing spoilage, but they realized that "Although spoilage of seafood is invariably attributed to the activity of contaminating microorganisms, loss of freshness, which often precedes microbial spoilage, primarily involves autolytic reactions controlled by endogenous enzymes present in the muscle tissue as well as those leaking from the gut. ATP is the main source of energy."

2.3. ATP breakdown products and *K* value as freshness indicator

The breakdown of ATP in fish is presented in Figure 2. As stated in the Introduction, certain nucleotide products could be used as a freshness indicator. Tarr (1966) considered the possibility that RNA might be a precursor of ATP. However, experiments indicated that RNA was not degraded in fish muscle at 0^{o}C. Very small amounts of IMP may arise through the degradation of nicotine adenine dinucleotide (Tarr 1966).

One of the ATP decomposition products, Hx, has been solely used as a freshness indicator (Beuchat 1973, Ehira & Uchiyama 1973, Jahns *et al.* 1976 and Jhaveri *et al*. 1982). Hattula and Kiesvaara (1982) determined Hx during the storage of vendace, perch, whitefish, and rainbow trout. Hx increased slowly at +10 - +14^{o}C, but the increase of Hx was really very slow at 0^{o}C. Gill (1995a) reviewed the changes of Hx during chill storage and found that there is a lot of variation between fish species (Fig. 3).

Ehira & Uchiyama (1986) found that some fish species produce more Ino than Hx while other fish species produce more Hx than Ino.

Williams *et al.* (1991) analyzed 20 tropical fish species for Ino and Hx and found that 11 of them were classified as inosine producers and five as hypoxanthine producers.

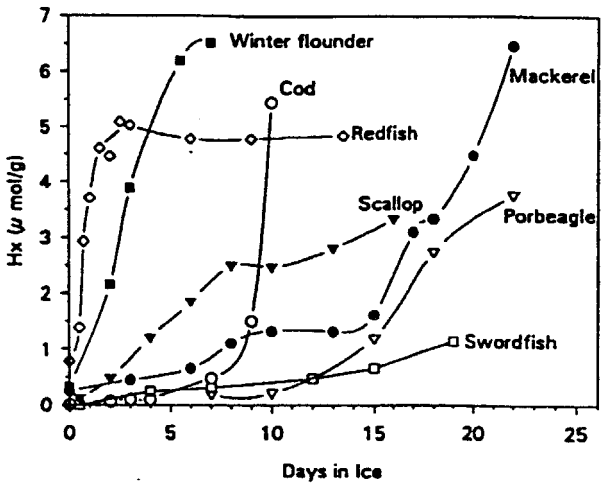

Figure 3. Variation in the rate of Hx accumulation of several species during storage in ice (Gill 1995a, adapted from Fraser et al. 1967).

The *K* value is more universal than determination of a single compound like Hx. Originally, the *K* value was defined by Saito *et al.* (1959a).They suggested that $K = 100 * B/A$, where *A* is the total absorbance at 250 nm of a perchloric acid extract and *B* is the absorbance of inosine and hypoxanthine at the same wavelength. Inosine and hypoxanthine were isolated by using ion exchange chromatography. The *K* value is defined generally by the following formula (Gill 1995a):

$$K \text{ value } (\%) = \frac{[\text{Ino}]^* + [\text{Hx}]}{[\text{ATP}] + [\text{ADP}] + [\text{AMP}] + [\text{IMP}] + [\text{Ino}] + [\text{Hx}]} \times 100 \quad [2]$$

* [ ] molar concentrations

Luten *et al.* (1988) found that there was a linear correlation between *K* value of sole (*Solea solea*) and storage time as well as between sensory score and *K* value.

Handumrongkul and Silva (1994) proposed a modified *K* value (K^*). The formula is similar to formula [1] except that Ino is excluded. Their K^* value was a better index than the traditional *K* value for hybrid striped bass (*Morone saxatilis x Morone chryops*), because the concentration of Ino fluctuated during storage.

Luong *et al.* (1992) defined the hypoxanthine ratio or *H* value as:

$$H = \frac{Hx}{IMP + Ino + Hx} \quad [3]$$

Hilz *et al.* (1971) reviewed the applicability of certain quality indices on certain fish species. They found that the reaction: IMP > Ino > Hx is common to a large variety of British, American, Japanese and Canadian fish species. They showed that the rate of Hx increases rapidly during storage in redfish (*Sebastes marinus*) and winter flounder (*Pseudo-pleuronectes americanus*), but very slowly in swordfish (*Xiphias gladius*). They also found that the activity of IMP phosphohydrolase is very high in redfish, winter flounder and plaice (*Hippoglossoides platessoides*) compared to that in cod (*Gadus morhua* L). Yokoyama *et al.* (1994) used the Hx/AMP ratio as a freshness index for spear squid, because it was a better index in this case better index than the traditional *K* value.

Ehira and Uchiyama (1986) compared different biochemical and microbial changes in relation to freshness. The *K* value, as well as TMA-N, increased at the same speed in aseptic and non-aseptic muscle of Pacific cod (*Gadus macrocephalus*) and yellowtail (*Seriola quinquequeradiata*). This means that *K* value changes are caused by of muscle enzymes and not because of microbes. They examined 34 Japanese fish species and found rather large differences between fish species in days during which *K* values reached 20%. Ehira and Uchiyama (1986) proposed this limit as a good quality limit for sashami and for ingredients for a special dish, sushi, in Japan. Nonetheless, Ehira and Uchiyama found a better correlation between fish freshness and the *K* value than between fish freshness and trimethylamine nitrogen (TMA-N) or total volatile basic nitrogen (TVB-N), when they analyzed 104 classified fish samples sold in the Japanese market. The best class was fish immediately killed (the first class). The second class was good quality sushi and the third class was medium quality sushi.

Williams *et al.* (1993) examined nucleotide degradation profiles of iced barramundi (*Lates calcifer*) and Nile perch (*Lates niloticus*) They proposed a *K* value of <30% to indicate excellent quality. Chiou *et al.* (1995) studied the spoilage of milkfish (*Chanos chanos*) at different temperatures. They studied changes in nucleotides and related compounds. They regarded the *K* value as a reliable freshness indicator and a value of 30% was proposed as the limit of acceptability.

The *K* value can also be used as a freshness criterion of pickled fish. The *K* value increases the more rapidly salt content is lowered and the higher the temperature (Ponce de Leon *et al.* 1994). Because ATP decomposes very

quickly to IMP (Tarr 1966), Karube *et al.* (1984) simplified the definition of *K* value by excluding the adenosine phosphates. Karube's K_1 value is defined as follows:

$$K_1(\%) = \frac{[\text{Ino}] + [\text{Hx}]}{[\text{IMP}] + [\text{Ino}] + [\text{Hx}]} \times 100 \quad [4]$$

Karube *et al.* (1984) also proved that the correlation between the traditional *K* value and K_1 was good when the test material species were sea bass (*Lateolobrax japonicus*), saurel (*Trachurus japonicus*), mackerel (*Sxcomber japonicus*) and yellowfish (*Seriola quinquediata*).

Bremner *et al.* (1988) found that the shelf-life of tropical species of fish was related to the rate of IMP breakdown rather than to bacterial spoilage. Tropical fish are claimed to have an extended shelf-life on ice. A reason for this phenomenon is that there are mesophilic microbes instead of psycrophilic microbes in tropical fish and mesophilic microbes get a shock at cool temperatures, for example when ice is added. Another reason for the good keepability of tropical fish is that, at tropical temperatures, there is considerable washing when a lot of water from melting ice rinses the surface of fish.

Hara and Uda (1984) proposed a model relating *K* values to the rate of IMP breakdown by autolysis, based on a first-order reaction mechanism according to the following equation:

$$K_t = 100 - (100 - K_0)e^{-kt} \quad [5]$$

where K_t and K_0 are *K* values at times *t* and the time when IMP is at its estimated maximum value, and *k* is the rate constant for different tropical fish species.

2.4. ATP breakdown products in salted fish products

Ponce de Leon *et al.* (1994) examined the shelf-life of sardine (*Sardinopsis melanosticia*) and found that the lower the salt content the faster the *K* value increased. The salt content varied from 4 to 15%.

Oba & Niwa (1992) found that 0.5 M salt suppressed degradation of IMP to Ino during storage, and in ground sardine NaCl and KCl had a stronger effect on suppression than $CaCl_2$. Later on Oba & Niwa (1993) purified two

enzymes involved in IMP degradation from walleye pollack (*Theragra chalcogramma*) and silver whiting (*Sillago japonica*). They found that the 5'-nucleotidase was inhibited by NaCl and very strongly by $CaCl_2$. Purine nucleoside phosphorylase from silver whiting was inhibited by salts (NaCl, KCl, $MgCl_2$, and $CaCl_2$), but that of walleye pollack was not inhibited by NaCl or $CaCl_2$.

Makarewicz (1969) purified AMP-aminohydrolase from the muscle of the Thomback ray (*Raja clavata*) and found that the activity of the enzyme decreased 50% in a 0.6 M KCl solution.

2.5. ATP breakdown products as flavour components

Konosu and Yamaguchi (1982) reviewed the flavour components of fish and shellfish. They regarded nucleotides as important taste-affecting compounds. A relatively high IMP concentration is typical for bonefish while in crustaceans and mollusca, AMP and ATP are the dominating nucleotides. Fuke (1994) reviewed the taste-active compounds in seafood. According to him, the degradation of ATP to AMP and/or to IMP is very quick. IMP and AMP are the main taste-active compounds in cooked crabs. Fuke (1994) also examined the taste-active compounds of 'Katsuobushi', which is a special Japanese dish made of skipjack (*Dorosoma cepedianum*). He found that the key components in eliciting the specific taste were glutamic acid, histidine, lysine carnosine, IMP, inosine, hypoxanthine, creatine, lactic acid, sodium, and potassium ions. Originally Kodama identified the histidine salt of IMP as the substance responsible for the umami taste of 'Katsuobushi' (Kuninaka *et al.* 1964).

It has been proposed that Hx is one compound which causes a bitter off-taste (Jones 1967). On the other hand, the association of bitter flavour with Hx has been questioned (Hashimoto 1965; Tarr 1966).

Kuninaka *et al.* (1964) proposed that only the 5' IMP isomer is an active flavour potentiator, not the 2' or 3' isomer. IMP worked together with L-glutamate, not with the D-isomer. Kunimoto *et al*. (1993) reported that IMP, together with free amino acids, enhanced the flavour of the kipper of round herring (*Etrumeus teres*).

Spinelli & Miyauchi (1968) stored irradiated English sole (*Parophrys vetulus*), Pacific ocean perch (*Sebastes alutus)*, and petrale sole (*Eopseta jordani*) in ice until IMP had been decomposed. After the treatment, IMP

was added at different levels to the muscle homogenates of each species in order to restore the fresh taste. It was found that the flavour threshold of IMP was approximately 0.7 mmol/kg.

Murata and Sakaguchi (1989) treated yellowtail (*Seriola quinquecradiata*) with phosphatase, which decomposed IMP, and found a remarkable decline in flavour intensity. The addition of IMP at 0.5 mmol/kg resulted in significant recoveries of all flavour attributes. When 2 mmol/l of IMP were added, the fish extract showed the maximum flavour intensity; higher levels of IMP did not enhance the intensity any further. Murata & Sakaguchi (1989) studied five flavour attributes; 'umami', sourness, thickness, fresh fish favour, and overall taste quality. 'Umami' is characterized as a basic taste by the Japanese and is defined as the common taste caused by monosodium glutamate and IMP. Conn (1992) has called 'Umami' as 'the fifth basic taste'. According to him, monosodium glutamate (MSG) is used alone to impart umami, whereas nucleotides require a source of MSG to act as a flavour enhancer. Although the umami taste has been accepted in Japan as a basic taste, it is not accepted in Europe and America (Fuke, 1994).

When Bremner *et al.* (1988) studied the keepability of tropical fish it was found that the IMP level was related to both flavour intensity and acceptability.

Apparently the determination of ATP breakdown product suits the evaluation of fish freshness, when high demands for freshness, as in Japan and Australia, are proposed (Ehira & Uchiama 1986; Williams *et al.* 1993). It is not, however, relevant to establish any concentration limits of ATP breakdown products for acceptable fish, because there are variations in breakdown products between fish species and even between individual fish.. In some fish species, the *K* value increases faster than the sensory changes are detected. Luong *et al.* (1992) revealed that the *K* value of Pacific cod stored on ice approached 100% after 2 days yet the fish were judged suitable for consumption up to 10 days later. Greene *et al.* (1990) found that the *K* value of flathead sole was 80% after only 1 day in ice.

2.6. Determination of ATP breakdown products

Kennish and Kramer (1987) reviewed different methods for measuring nucleotide degradation in fish muscle. An early method separated the nucleotides from nucleosides and bases present in fish muscle extracts by fractionation as barium salts. The most general extraction technique has

been perchloric acid (PCA), but trichloroacetic acid (TCA) and the acetonitrile technique have also been used (Kennish & Kramer, 1987). Chunyan *et al.* (1995) determined IMP by paper chromatography.

The original method of Saito *et al.* (1959a) is based on ion exchange chromatography. Recently, the HPLC technique has replaced the time-consuming ion exchange chromatography. Gill (1992) reviewed different biochemical and chemical indices of seafood quality. A lot of rapid methods have been presented, including electronic biosensors and immobilised enzyme test strips. These technologies have been patented, but have not achieved wide commercial acceptance. The enzymatic method by which Karube *et al.* (1984) determined K_1 value (formula 3), is based on the oxygen consumption catalyzed by the last enzyme, in the chain IMP > Ino > HX > (xanthine oxidase) uric acid (U). IMP was decomposed to Hx by a membrane containing 5' nucleotidase (EC 3.1.3.5 from *Crotalus adanteus*-venom), nucleoside phosphorylase (EC 2.4.2.1. from calf spleen) and xanthine oxidase (EC 3.1.3.3.5. from bovine spleen). The oxygen consumed by the reaction chain was determined by oxygen electrode. Shen *et al.* (1996) developed a hypoxanthine biosensor using the almost identical principle.

Gill (1992) stated that the most commonly used alternative to the analysis of nucleotides has been HPLC. Perhaps the most common method is that of Ryder (1985). He analyzed IMP, Ino and Hx from deproteinized fish extracts in less than 15 min using a C-18 reverse phase column (Gill 1995b).

Nguyen *et al.* (1990) separated IMP, Ino and Hx of fish tissues using capillary electrophoresis.

Luong *et al.* (1992) showed that two procedures, the determination of K_1 value (formula 3) by enzyme assay and the *H* value (formula 2) using capillary electrophoresis, agreed for trout, salmon and cod. The *H* and *K* values increased with storage time.

Chiba *et al*. (1991) presented a method for quality evaluation of fish using ^{31}phosphorus- nuclear magnetic resonance. It is probably only suitable for the detection of the initial decrease in freshness, because the high energy phosphates, like phosphocreatine a and different isomers of ATP are determined by that method.

Luciferase of the American firefly (*Photinus pyrarlis*) has been used for the rapid determination of ATP in fish muscle. The determination of ATP solely is not relevant for freshness studies, because ATP breaks down quickly after the fish death.

3. Aims of present study

Although sensory evaluation is a crucial means of measuring the quality of fish, it demands many persons and the training of personnel. The need for the development of rapid, simple and objective methods for assessing commercially interesting parameters of quality is obvious (Sorensen 1992). Therefore, it will be useful to understandthe chemical changes related to the loss of freshness. After clarification of the chemical changes rapid methods could be developed.

The *K* value is still questioned because of individual variation between species, due to sampling and the degree of dark and white muscle. Also the dependency on catch area, gear, season and weight of fish seem to influence the estimation of *K* value. (Sorensen 1992).

The aim of this study was to reveal whether ATP breakdown products and the *K* value calculated from the breakdown products could be used as a freshness indicator of certain Finnish species and fish products. In addition to these aims, the applicability of *K* value to measure the effect of catching method was undertaken. Because there is a need for rapid methods in the fish industry, the applicability of one rapid method was tested.

4. Materials and methods

A detailed description of the experimental procedures is presented in the original papers I -V.

The fish species examined are listed in Table 1.

Table 1. The fish species examined in Publications I - V.

Fish species	Publication
Baltic herring (*Clupea harengus membras*)	III, IV, V
Pike (*Esox lucius*)	I
Sea trout (*Salmo trutta*)	I
Bream (*Abramis blicca*)	I
Flounder (*Pleuronectes flesus*)	I
White bream (*Abramis blicca*)	I
Vimba (*Abramis vimba*)	I
European whitefish (*Coregonus wartmanni*)	II
Whitefish (*Coregonus lavaretus*)	IV
Rainbow trout (*Oncorhyncus mykiss*)	IV
Mackerel (*Scomber scombrus*)	IV

4.1. Preparation of samples

Frozen pieces taken from muscle were mainly used as the samples for the determination of ATP (Publications I, II, III, IV, and V). After catching or storage for certain times, fish muscles were frozen and transported to a freezer (temperature –20^{o}C). Before freezing, fish were gutted and placed in Minigrip plastic bags. The temperature of the frozen storage was –25^{o}C. Within 2 months, frozen fish samples were extracted and analyzed according to Ryder (1985).

For the examination effects of different sample preparation the pike extracts were prepared by three different methods:

(1) Unfrozen fish muscle was extracted with 0.6 M perchloric acid and analyzed immediately (PCA method).
(2) Fish muscle was frozen with solid carbon dioxide ice and the extraction with PCA was done using thawed muscle (CO_2 method).
(3) Fish muscle was frozen with liquid nitrogen and the extraction with PCA was done using thawed fish muscle.

For the examination of the effects of salting on the ATP breakdown, rainbow trout (*Oncorhyncus mykiss*) and whitefish (*Coregonus wartmanni*) were salted in a saturated salt solution (26% (w/w) NaCl) and in a so-called 'gravad' salt solution (14% NaCl (w/w) and 1.4% saccharose (w/w). The ratio of fish and solution was 1:4. Salted fish were stored at +5^{o}C for a month. The samples were taken periodically by freezing 20 g of fillet.

4.2. Methods

ATP breakdown products were determined by high performance liquid chromatography (HPLC) according to Ryder (1985) with the exception of sample preparation. The coefficient of variation of quantitation of ten replicate standard injections varied, according to Ryder (1985), between 0.93 and 1.89%.

All the samples were frozen and thawed before extraction with perchloric acid solution. The identification of different compounds was based only on the retention times (I, II, III and V). When analyzing ATP breakdown products of heated fish products (Publication IV) the identification was based on ultraviolet spectra (diode array detector) in addition to retention times. The samples were frozen in dry ice or by putting samples one by one into frozen storage.

In Publication V, a rapid paper strip method was used in addition to the HPLC method. The rapid paper strip method (Negishi & Karube 1989) is based on the measurement of concentrations of IMP, as well as concentrations of Ino and Hx, where a sheet of filter paper is soaked in a solution containing the two kinds of enzymes, NAD, iodonitrotetrazolium chloride, and the inhibitor of lactate dehydrogenase; this procedure was followed by freeze-drying. Apparently a similar paperstrip method has been commercialized by a French firm, Transia. By this rapid method soaking the strip in the buffer extract from fish causes a colour change, which is compared to a colour chart. The red hue changes to yellow when IMP (inosinic acid) concentration decreases and the white hue changes to red when the concentration of Ino and Hx increases.

When comparing the differences in ATP breakdown of Baltic herring in different seasons, the methods were similar to that in Publication III.

In Table 2 the different monitoring methods are summarized.

Table 2. The methods for evaluation of freshness in addition to ATP breakdown products.

Method	Publication
Sensory evaluation Torrymeter Gr Rigor mortis *Rigor mortis*	II, III, V II, III III

5. Results and discussion

The main detailed results are presented in the original papers I - V.

5.1. The effect of sampling on the recovery of ATP

Degradation of nucleotides does not occur when fish is in the frozen state (Raja & Moorjani, 1971).

Table 3 gives the results of three different methods, direct 0.6 M perchloric acid (PCA method) extraction, freezing of sample by liquid nitrogen (LQN method) and by dry carbon dioxide ice (CO_2).

Table 3. The concentrations of ATP and its breakdown products in pike (Esoxlucius) immediately after killing, prepared by three different methods: (1) DPCA = direct 0.6 M perchloric acid extraction, (2) LQN = frozen by liquid nitrogen before extraction with PCA, and CO_2 = Frozen with dry carbon dioxide ice before extraction with PCA. (The numbers are means of two or three determinations)

Method	*K* value	ATP	ADP	AMP	IMP	Ino	Hx
	%	mmol/kg					
DPCA	8.0	4.4	1.8	0.6	3.3	0.6	0.2
LQN	5.9	2.4	1.5	0.5	3.3	0.4	0.5
CO2	10.3	0.6	0.6	0.6	5.4	0.7	0.1

The *K* values varied between 5.9 and 10.3%, but the largest variations were between the concentrations of ATP and IMP. It is remarkable that when freezing the sample with carbon dioxide ice, there is a lot of IMP and very low concentrations of adenosine phosphates. This phenomenon explains why not a lot of adenosine phosphates were found (Publications I - V). This should not effect the *K* value very much, because the concentrations of ATP, ADP, AMP and IMP are denominators when calculating the *K* value. This should reveal that all the adenosine phosphates turn to IMP during freezing and thawing of the fish sample.

In Publications I - V the samples were frozen at –20^{o}C before extraction with 0.6 M perchloric acid. The dephosphorylation of ATP occurs very

quickly after killing of fish (Jessen, 1995) in chill storage (Kennish & Kramer, 1987). Williams *et al.* (1993) examined the ATP breakdown products of frozen barramundi and Nile perch. They found that the nucleotides ATP, ADP and AMP were not present in any significant amount in any of the samples; all concentrations were below 0.5 mmol/kg. Degradation of ATP also occurs especially during freezing and thawing of fish muscle (Jessen, 1995). The skin of fish contains appreciable quantities of hypoxanthine and guanine (Hilz *et al.* 1971) and therefore the skin was removed before analysis. There was no ATP at all when frozen and thawed samples were analyzed.

The anatomical location of sampling was studied with whitefish (Publication II, Table 1). There were no differences related to anatomical location. Kanoh *et al.* (1986) got similar results. Love (1974) discussed the importance of sampling of fish muscle. For example, the content of DNA is lower in the middle of the cod fillet than in either end. In the muscle of the European whitefish (Publication II), there was a minimal proportion of dark muscle.

Kanoh *et al.* (1986) found that the IMP content was much higher in the ordinary muscle of certain tuna fishes (*Thunnus alalunga*, *Neothunnus albacore*, and *Katsuwonus pelamis*) than in the red muscle. Saito *et al.* (1959b) also found that there is less IMP in the red lateral muscle of some fishes (mackerel, trout and rainbow trout) than in dorsal muscle. In the muscle of the European whitefish there was a minimal proportion of dark muscle.

Love (1974) reviewed the differences between dark and white muscle and stated that there is no difference in ATP content between dark and ordinary muscle.

According to our studies, (Publication II, Table 1) there were, however, considerable differences between individual fish of the same catch. All the three whitefishes were from the same catch. Two fishes having Torrymeter reading 12, had *K* values of 35% and 43%. A fish having Torrymeter value 10 had a *K* value of 69%.

5.2. The effect of fish species on the ATP breakdown products

In the first publication (I) the ATP-related compounds were studied in six different fish species from the Baltic sea. The ATP breakdown products of whitefish are presented in Publications II and IV. The nucleotide-related compounds of Baltic herring are presented in Publications III, IV, V and in Table 5. In sea trout and rainbow trout the increase in *K* value and the decrease of IMP occurs slower than in other species. The rapid increase in *K* value and rapid decrease of IMP in pike (Publication I) may be explained by the inclination of pike to jerk in the gillnet or to an unexplainable illness of the examined pike.

There were a lot of variations between the species. This phenomenon is well known in the earlier studies (Hilz *et al*. 1971; Ehira & Uchiyama, 1986). Marseno *et al.* (1992) found that 5'-nuclotidase activity varied remarkably between four fish species (*Sebastes inermis*, *Stephanolepsis cirrhifer*, *Nemipterus virgatus*, and *Parapristipoma trilineatum*). Kemp & Spinelli (1969) compared the rates of IMP degradation in unfrozen and frozen–thawed (slacked) fish and found that there were no differences. In that context, they found different rates for different fish species. The species species were English sole (*Parophrys vetulus*), Dover sole (*Microstomus pacificus*), rainbow trout (*Salmo gairdneri*), silver salmon (*Oncorhyncus kisuttch*) and Pacific halibut (*Hippoglossus stenolepsis*). The rate of IMP degradation was fastest in Dover sole and slowest in halibut (Kemp & Spinelli, 1969).

When we found that the degradation of IMP was very rapid in pike (*Esox lucius*) (Publication I) another experiment was performed. Later on we conducted an experiment with five pikes. The pikes of this experiment were kept in a receptacle made for keeping fish alive for 2 weeks before killing. The results of the determinations of the ATP breakdown products are given in Table 4.

Table 4. The K values and IMP concentrations of pike stored in ice and at +5°C. The means and standard deviations are of five individual fish.

Temperature °C	Days	*K* value %	IMP mmol/kg
0	1	14 ± 6	7.0 ± 0.9
5	1	18 ± 9	6.6 ± 1.1
0	4	40 ± 11	4.8 ± 1.0
5	4	48 ± 7	3.9 ± 0.9
0	7	56 ± 20	3.2 ± 1.7
5	7	71 ± 12	2.0 ± 1.2

In the experiments reported in Publication I, the pike was caught by gillnet, which was examined once per week during wintertime. The reason of the differences could be the rest before killing, the above mentioned unknown illness of the examined pike, or the catching method (the fish was caught by gillnet, Publication I). It has been found that there are quite a lot of variations in chemical indices affected by the physiological state of wild fish (Borresen, 1992; Botta, 1994).

In spite of the differences between different fish, *K*-value increases almost linearly in all fishes (Publication I: Fig. 1B; Publication II: Fig. 3A; Publication III: Fig. 3, Tables 4 and 5).

Table 5. The K values and ATP breakdown compounds of Baltic herring during storage in ice and at +4°C. Mean values and standard deviations are from five individual fish.

Days	Temperature °C	*K* value %	IMP	Ino	Hx
			mmol/kg		
0	0	27 ± 8	6.4 ± 0.2	1.9 ± 0.6	0.7 ± 0.3
4	0	60 ± 9	3.1 ± 1.0	3.5 ± 0.5	1.6 ± 0.5
4	+4	80 ± 9	1.6 ± 1.0	4.5 ± 0.0	3.5 ± 0.8
7	0	83 ± 9	0.7 ± 0.6	2.8 ± 0.6	2.4 ± 0.9
7	+4	93 ± 3	0.3 ± 0.2	2.8 ± 0.6	5.6 ± 0.9

The ATP breakdown of Baltic herring caught by trapnet during chill storage are presented in Table 5. In some cases the curve is hyperbolic (Publication I: Fig. 1A, and Publication II: Figs. 3, 4 and 5).

5.3. Relationships between ATP breakdown products and sensory analysis

The sensory analysis of whitefish (*Coregonus wartmanni*) (Publication II) and Baltic herring (Publication V) was performed simultaneously with the determination of ATP breakdown products. The sensory quality of whitefish was examined in four seasons. The sensory quality was relatively good after 7 days in ice and 5 days at +5°C (Publication II, Table 5). During these periods the *K* value remained below 80% and the IMP concentration was over 1.0 mmol/kg in all other seasons except in spring (May) (Publication II, Figures 2, 3 and 4).

The results obtained from experiments with Baltic herring showed the *K* value determined by the paper strip method was related to sensory quality of fish (Publication V). However, the *K* value determined by HPLC was not well related to *K* value determined by the paper strip method. The *K* values determined by paper strip method were smaller than those determined by HPLC.

5.4. Effect of temperature during storage on the degradation of ATP-related compounds

The higher the rate of the degradation of ATP-related compounds the higher the temperature (Publication II: Fig. 2, Tables 4 and 5). Similar results were obtained by Japanese researchers when studying the spoiling of plaice (*Paralichthys olivaceus*) (Iwamoto *et al.* 1987), bonito (*Sarda* .spp.) (Oka 1989), and sardine (*Sardinops melanosticta*) (Ponce de Leon *et al.* 1994). Iwamoto *et al.* (1987) found that the ATP concentration of plaice muscle increased faster at 0^{o}C than at +5 ^{o}C even though the increase in the *K* value was slower at 0^{o}C than at +5 ^{o}C. Iwamoto *et al.* (1987) also found that the onset of rigor mortis *rigor mortis* started later at +5 ^{o}C than at 0 ^{o}C. The muscle of plaice showed full rigor when ATP completely disappeared and lactic acid attained the maximum plateau.

5.5. ATP breakdown products as quality criterion when studying the effects of catching method on fish quality

The effects of catching method on certain quality indices of Baltic herring were examined (Publication III). The changes in ATP breakdown products were a little different for the three methods examined. The *K* value was, in

some cases, the largest fish caught by gillnet (Publication III: Fig. 3. and Table 6), then in the fish caught by trap and trawl, respectively.

Table 6. The K values and ATP breakdown products of Baltic herring caught by different methods. Fish was caught in May 1992. The means were calculated for three different harvests. 2 h and 4 h are the duration times of trawling in hours.

Method	*K* value	IMP	Ino	Hx	Temperature of fish
	%	mmol/kg			^{o}C
Trawl, 2 h	21	6.6	0,2	1.7	9.6
Trawl, 4 h	20	6.2	0.5	1.3	8.8
Gillnet	30	5.5	1.3	1.3	11.4
Trap	25	6.5	0.6	1.8	14.1

The duration of trawling did not remarkably affect the ATP breakdown products. Botta and Squires (1983) assessed the sensory quality of cod (*Gadus morhua*) caught by different methods. The quality of fish caught by gillnets set 1 and 2 days was moderately inferior to the fish caught by trap or by baited hook. When gillnets had been set for 3, 4, or 5 days the overall sensory quality was greatly inferior to that of cod caught by baited hook or trap. In our studies gillnets were examined daily.

Botta *et al.* (1987a) found that the gillnet-caught cod was different from those caught by handline, longline or trap. The method of catching significantly affected moisture and protein content of Atlantic cod. Botta *et al.* (1987b) also found that the method of catching affected colour and sensory variables.

In contrast to the studies of Botta *et al.* (1987b), we did not find differences in the sensory quality of Baltic herring caught by different methods when studying the sensory quality by triangle test (Publication III, Table 4).

Love (1974) discussed the effects of stress on chemical results. 'What are the 'true' chemical values in any case? Are they the concentrations characteristic of gentle swimming without undue stress?' Capture by net, hook or trawl involves a desperate struggle for the fish.

Understandably Love (1974) found that the levels of adenosine phosphates (ATP, ADP, and AMP) were higher in cod muscle before exercise than after

exercise. Consequently, the level of IMP was lower before exercise than after exercise.

Generally the differences caused by the catching method were less than lay persons have supposed, when the time of dying was corrected for, e.g. as regards the gillnet, catches, according to present practice, are iced about 2 h after removing from the net.

5.6. The effect of season on the *K* value and IMP concentration of whitefish and baltic herring

The *K* value of whitefish was smallest at the beginning of storage, when fish were caught in the late summer (Publication II: Fig. 3). Correspondingly, the IMP concentration at the start of storage was the highest, when comparing the effect of season on ATP-related compounds (Publication II: Fig. 4).

The IMP concentration of Baltic herring was at the highest level in September (Table 7) and at the lowest level in April. The *K* value was the highest in May.

Table 7. The K values and IMP concentrations of Baltic herring caught in different seasons of the year by trawl. The mean values and standard deviations are from three individuals. The duration of hauls was 2 h.

Date	*K* value %	IMP concentration mmol/kg
2 April 1992	17 + 8	5.6 + 0.5
25 May 1992	26 + 13	6.6 + 0.8
17 September 1992	20 + 3	7.8 + 0.5
12 November 1992	20 + 5	6.5 + 0.7

The high IMP value could indicate a good condition of fish. It is well known that the fat content of Baltic herring is higher in autumn than in spring, when the fish is spawning. The IMP content of Baltic herring acquired from retail shops was also higher in the autumn than in early summer (Kiesvaara *et al.* 1992). Correspondingly, the *K* value was higher in the summer than in autumn.

It seems probable that the IMP (= ATP) level *post mortem*, depends on the food intake. When the fish has eaten during summer and autumn the

nucleotide levels are at their highest. The fat content of Baltic herring is at its highest between August and November and at its lowest in June (Sjöblom 1961).

5.7. ATP breakdown products in heated fish

IMP does not decompose totally during a short heating such as smoking or *sous vide* treatment (Publication IV). The rate of ATP degradation depends, however, on the fish species.

IMP is apparently an important flavour potentiator in cooked fish. Thomson *et al.* (1980) reported the IMP concentration of cooked halibut (*Gadus aeglefinus*) to be 2.89 mmol/kg. They also conducted an experiment to prove the importance of nucleotides and amino acids as flavour components. The overall flavour intensity (scale 0 = absent to 5 = very strong) of amino acid and nucleotides at the same concentration as in cooked haddock were 1.79 and 1.75, respectively, when tasted separately, but when amino acids and nucleotides were put together, the flavour intensity was 2.58.

Even though the enzymes breaking down ATP and ATP-related compounds are being studied, there is little known about the heat stability of the enzymes. Tarr (1955) isolated riboside hydrolase from fish and found that at 50 and 55 ^{o}C hydrolysis was very rapid but the enzyme soon became inactive. Nedachi & Hirota (1992) purified 5'-nucleotidase from snapper muscle and found that the activity of the enzyme decreases at +40 ^{o}C and ceases at +60 ^{o}C. When fish is heated there are two accelerated reactions acting simultaneously, the decomposition of nucleotides and the denaturing of the enzymes causing the nucleotide breakdown. After the conventional smoking of rainbow trout and during the *sous vide* treatment of whitefish there was still so much IMP left that it had an effect on taste (Publication IV and Murata & Sakaguchi, 1989).

The enzymes degrading ATP breakdown products of rainbow trout were inactivated between 60 and 70 ^{o}C (Publication IV, Fig. 4). The hypoxanthine producing enzyme, ribohydrolase, denatured at higher temperature than the other enzymes examined. According to Surette (1987) ribosehydrolase in fish originates from bacteria. Some bacteria are quite temperature tolerant and this could explain the different behaviour during the heating process.

5.8. ATP breakdown products in salted fish

The *K* value of whitefish in the saturated NaCl solution increased slower than in the diluted salt solution (Fig. 4).

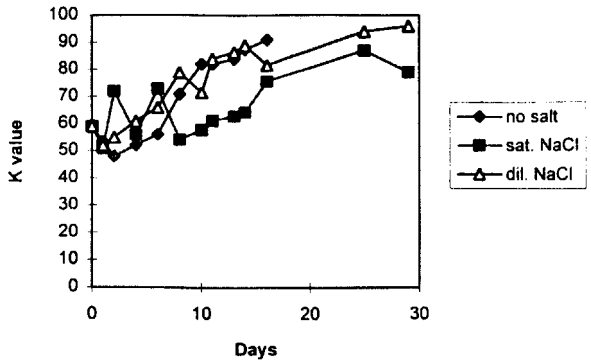

Figure 4. The changes of K value in salted and non-salted whitefish during storage at +5 °C.

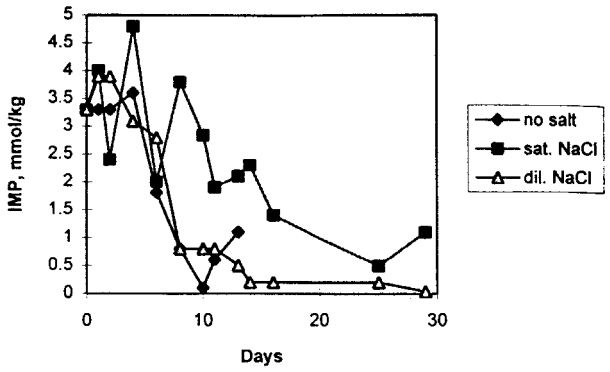

Figure 5. The changes of IMP concentration in salted and non-salted whitefish during storage at +5°C.

In contrast the concentration of IMP decreased faster in the saturated salt solution than in the diluted salt solution (Fig. 5). In the case of rainbow trout differences between dilute and strongly salted fish were not found (Figs. 6 and 7).

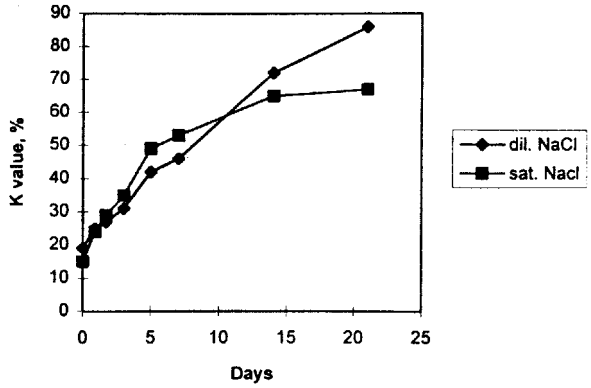

Figure 6. The changes of K value in salted rainbow trout during storage at +5°C.

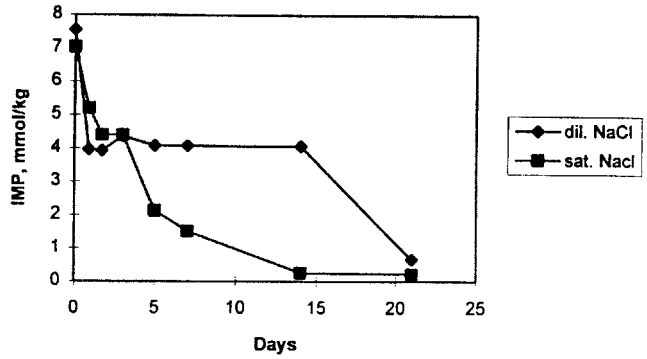

Figure 7. The changes of IMP concentration in salted rainbow trout during storage at +5°C.

There was an increase in dry weight during the first days (Table 8).

Table 8. The relative changes of dry weight of salted rainbow trout in saturated salt solution and in 14 % (w/w) salt solution.

Time (days)	RDW* In saturated salt solution	Salt in fish %	RDW In 14 % salt solution	Salt in fish %
0	100		100	
1	119	4.8	115	1.8
2	134	10.0	106	5.3
3	122	11.2	100	6.8

*RDW = relative dry weight

This indicated that the liquid comes out of muscle at the beginning of storage. This phenomenon explains the decrease in IMP concentration on the second day (Fig. 6).

5.9. Relationships of the onset of *rigor mortis* and *K* value with torrymeter reading

In this study correlation coefficients were not determined. However, during storage, other methods in addition to *K* value were used. In Publication II it was found that whitefish individuals which had a low Torrymeter reading had a lower IMP content.

In Publication III the IMP content decreased faster in the fish caught by gillnet than in fish caught by other devices. The onset of rigor mortis *rigor mortis* started sooner in the fish caught by gillnet than in fish caught by other means. This indicates that the stress shortens the time of the onset of *rigor mortis*. Korhonen *et al.* (1990) found that the ATP/IMP ratio was higher in non-stressed fish (*Areochromis aureus*) than in stressed fish.

Sakaguchi and Koike (1992) studied skin on fillets from six fish species (*Sardinops melanosticus, Seriola quinqueradiata, Pagrus major, Paralichtys olivaceus, Navodon modestus, Plecoglossus altivelis*) for freshness using the Torrymeter and *K* value. They concluded that the Torrymeter readings measured on the bone or skin side, depending on fish species, could be an indicator superior to *K* value for determining the freshness of

fish fillets in the earlier period of ice storage. However, the *K* value increased, linearly but slowly during storage.

5.10. Applicability of rapid methods

Publication V presented results which indicated that the results attained by the rapid paper strip method correlated with the sensory analysis, but not well with the results attained by the HPLC method. Malle & le Pezennec (1992) found that correlation of *K* values with the rapid paper strip method with freshness index, based on organoleptic inspection, was high (correlation coefficients 0.93, 0.98 and 0.98 for salmon, mackerel, and whiting, respectively.

The *K* values attained by the paper strip method are not identical with those attained by HPLC. This does not, however, mean that the paper strip method is useless, if the paper strip method is correlated to the results obtained by sensory evaluation. The *K* values determined by the paper strip method were lower than the values attained by HPLC for salmon (*Salmo salar*). Both of the *K* values, however, increased during storage (Sivertsvik *et al.* 1996).

6. Conclusions and future prospects

The *K* value calculated from the breakdown products can be used as a freshness criterion for the Finnish fish species studied. *K* value increased during storage in all species, however at different speed in different individual fish. If the *K* value of raw fish is below 30%, it means that the fish has not died long and that it has been stored near 0 ^{o}C. If the *K* value is between 70 and 100%, it means that the fish has been dead at least 1 day and has been stored near the temperature of smelting ice, or the fish has not been stored at low temperature. The *K* value could be regarded as a natural time-temperature indicator.

The anatomical location does not affect the concentrations of ATP breakdown products. On the other hand, the physiological state affects the catabolism of ATP. The stress caused by catching method accelerates the catabolism of ATP and its breakdown products, but there could be other reasons, which are still unknown.

The *K* value is suitable for revealing differences between catching methods. More research should be done in order to obtain more reliable results. For instance, in the studies presented here, the effect of catch size when catching fish by trawler could not be examined.

The *K* value can be used as a criterium of freshness for material of heated fish products. The denaturation of enzymes occurs faster than the breakdown of ATP-related compounds in conventional heat treatments of fish. During cold smoking the temperature is not high enough to denature fish enzymes.

The rapid paper strip test could be applied to the determination of fish freshness, but it did not give similar results to the *K* value attained by HPLC. The paper strip method, however, correlated with the sensory quality of fish.

The concentration of IMP seems to be higher in autumn than during other seasons at least in whitefish and in Baltic herring. There is, however, need to make additional studies in order to clarify whether this phenomenon could be generalized to include all fish species.

References

Ashie, I.N.A., Smith, J.P. & Simpson, B.K. 1996. Spoilage and shelf-life extension of fresh fish and shellfish. Crit. Rev. Food Sci.Nutr., 36, pp. 87 - 121.

Beuchat, L.R. 1973. Hypoxanthine measurement in assessing freshness of chilled channel catfish (*Ictalurus punctatus*). J. Food Sci., 38, p. 453.

Borresen, T. 1992. Quality aspects of wild and reared fish. *In*: Huss, H.H., Jakobsen, M. & Liston, J. (eds.). Quality Assurance in the Fish Industry. Elsevier Science Publishers B.V. Pp. 1 - 17.

Borresen, T. 1995a. Muscle anatomy and function. *In*: Huss. H.H. (ed.). Quality and quality changes in fresh fish. FAO Fish.Tech. Pap. No. 348. Rome, FAO 1995. Pp. 12 - 14.

Borresen, T. 1995b. Chemical composition. *In*: Huss, H.H. (ed.). Quality and quality changes in fresh fish. FAO Fish. Tech. Pap. No. 348. Rome, FAO. 1995. Pp. 20 - 34.

Botta, J.R. 1994. Freshness quality of seafood: a review. *In*: F. Shahidi & J.R. Botta (Eds.). Seafood, Chemistry, Processing, Technology and Quality. Blackie Academic & Professional, An Imprint of Chapman & Hall, London. Pp. 140 - 167.

Botta, J.R & Squires, B.E. 1983. Effect of method of catching on the sensory quality of inshore-Newfoundland caught cod (*Gadus morhua*). Can. Tech. Rep. Fish. Aquat. Sci. (Inspection Division, Department of Fisheries and Oceans, St. John's Newfoundland, A1C 5X1) 1164. iv + 18 p.

Botta, J.R., Kennedy, K. & Squires, B.E. 1987a. Effect of method of catching and time of season on the composition of Atlantic cod (*Gadus morhua*) J. Food Sci, 52, pp. 922 - 924, 927.

Botta, J.R., Bonnell, G. & Squires, B.E. 1987b. Effect of method of catching and time of season on sensory quality of fresh raw Atlantic cod (*Gadus morhua*). J. Food Sci., 52, pp. 928 - 931, 938.

Boyle, D.L., Lindsay, R.C. & Stuiber, D.A. 1991. Adenine nucleotide degradation in modified atmosphere chill-stored fish. J. Food Sci. 56, pp. 1267 - 1270.

Bremner, H.A., Olley, J., Statham, J.A. & Vail, A.M.A. 1988. Nucleotide Catabolism: Influence on the storage life of tropical species of fish from the North west shelf of Australia. J. Food Sci., 53(1), pp. 6 - 11.

Chiba, A., Hamaguchi, M., Kosaka, M., Tokuno, T., Asai, T. & Chichibu, S. 1991. Quality evaluation of fish meat by ^{31}phosphorus- nuclear magnetic resonance. J. Food Sci., 56, pp. 660 - 664.

Chiou, T.-K., Yu, C.-L. & Shiau, C.-Y. 1995. Changes of freshness and components of milkfish during storage. (Abstract in FSTA 28, 3 R 13), Food Sci. Taiwan, 22(1), pp. 46 - 58.

Chunyan, Y., Zhiping, L., Sen, S. & Yingxu, W. 1995. A study on the amount of inosinic acid in the muscle of Songhuajiang river carps and the meat keeping time. Fish. Sci. Taiwan, 14(5), pp. 15 - 17.

Conn, H. 1992. 'Umami' the fifth basic taste. Nutr. Food Sci., 2, pp. 21 - 23.

Connell, J.J. 1980. Sensory and non-sensory assessment of fish. *In*: J.J. Connell (ed.). Advances in Fish Science and Technology. Fishing News Books Ltd, Farnham, Surrey, UK. Pp. 56 - 65.

Dingle, J.R. & Hines, J.A. 1967. Extraction and some properties of adenosine 5'-monophosphate aminohydrolase from pre rigor and post rigor muscle of cod. J. Fish. Res. Bd Can. 24, pp. 1717 - 1730.

Ehira, S. & Uchiyama, H. 1973. Formation of Inosine and Hypoxanthine in Fish Muscle during Ice Storage. Bull. Tokai Reg. Fish Lab., No. 75, pp. 63 - 73.

Ehira, S. & Uchiyama, H. 1986. Determination of fish freshness using the K value and comments on some other biochemical changes in relation to freshness. *In*: Kramer, D.E. & Liston, J.C. (Eds.). Seafood Quality Determination. Elsevier Science Publisher B.V., Netherlands. Pp. 185 - 207.

Fraser, D.I., Dingle, J.R., Hines, J.A., Nowlan, S.C. & Dyer, W.J. 1967. Nucleotide degradation monitored by thin-layer chromatography and associated post mortem changes in relaxed cod muscle. J. Fish. Res. Board. Can., 24, pp. 1837 - 1841.

Fuke, S. 1994. Taste-active components of seafood with special reference with reference to umami substances. *In*: Shahidi, F. & Botta, J.R. (eds.). Seafood, Chemistry, Processing, Technology and Quality. Blackie Academic & Professional. An Imprint of Chapman & Hall, London. Pp. 115 - 139.

Gill, T.A. 1992. Biochemical and chemical indices of seafood quality. *In*: Huss, H.H., Jakobsen, M. and Liston, J.C. (eds.). Quality Assurance in the Fish Industry. 1992. Elsevier Science Publishers B.V., Amsterdam. Pp. 377 - 388.

Gill, T. 1995a. Autolytic changes. *In*: Huss, H.H. (ed.). Quality and quality changes in fresh fish. FAO Fisheries Technical Paper. No. 348. Rome, FAO. Pp. 39 - 50.

Gill, T. 1995b. Biochemical and chemical methods. In: Huss H.H. (ed.). Quality and quality changes in fresh fish. FAO Fisheries Technical Paper. No. 348. Rome, FAO. Pp. 139 - 148.

Greene, D.H., Babbit, J.K., Rebbond, K.D. 1990. Patterns of nucleotide catabolism as freshness indicators in flatfish from Gulf of Alaska. J. Food Sci., 55, pp. 1236 - 1238.

Handumrongkul, C. & Silva, J.L. 1994. Aerobic counts, colour and adenine nucleotide changes in CO_2 packed refrigerated striped bass strips. J. Food Sci. 59, pp. 67 - 69.

Hara, A. & Uda, F. 1984. Theoretical and experimental studies in the time-temperature tolerance of fish K-value. Bull. Jpn. Soc. Sci. Fish., 50, pp. 1745 - 1756.

Hashimoto, Y. 1965. Taste-producing substances in marine products. *In*: R. Kreuzer (ed.). The technology of fish utilization. Fishing News (Books) Ltd, London. Pp. 57 - 60.

Hattula, T. & Kiesvaara, M. 1982. Kotimaisen järvikalan laadun määritys tuoreusmittarilla. Ympäristö ja Terveys N:o 4/1982, pp. 272 - 279. (In Finnish.)

Hebard, C.E. Flick, G.J. & Mertin, R.E. 1982. Occurrence and significance of trimethylamine oxide and its derivatives in fish and shellfish. *In*: Martin, R.E.. Flick, G.J., Hebard, C.E. & Ward, D.R. (Eds.). Chemistry & Biochemistry of

Marine Food Products. Avi Publishing Company Inc. Westport, Connecticut, USA. Pp. 149 - 304.

Hilz, D.F., Dyer, W.J., Nowlan, S. & Dingle, J.R. 1971. Variation of biochemical quality indices by biological and technological factors. *In*: H. Krauser (ed.). Fish Inspection and Quality Control. Fishing News (Books) Ltd, London. Pp. 191 - 195.

Huss, H.H. 1976. Konsumfisk - biologi, teknologi, kvalitet og holdbarhed. Dan. Vet. Tidsskr. 59, pp. 165 -175.

Iwamoto, M. Yamanaka, H., Watabe, S. & Hashimoto, K. 1987. Effect of storage temperature on rigor mortis and ATP degradation in plaice *Paralichtys olivaceus* muscle. J. Food Sci., 52, pp. 1514 - 1517.

Jahns, F.D, Howe, J.L., Coduri, R.J. Jr. & Rand, A.G. Jr. 1976. A rapid visual enzyme test to assess fish freshness. Food Technol., July, pp. 27 - 30.

Jason, A.C. & Richards, J.C.S., 1975. The development of an electronic fish freshness meter. J. Phys. E. Sci. Instrum. 8, pp. 826 - 830.

Jessen, F. 1995. Technological laboratory, Danish Ministry of Fisheries, Technological University, Lyngby, Denmark. Personal communications.

Jhaveri, S.N., Leu, S.-S. & Constantinides, S.M. 1982. Atlantic Mackerel (Scomber scombrus, L.): Shelf life in ice. J. Food Sci., 47, pp. 1808 - 1810.

Jones, N.R. 1967. Fish Flavours. *In* Schoultz, H.W. Day, E.A. & Libbey, L.M. (Eds.). Chemistry and physiology of flavours. Avi Publishing Co. Westport, Connecticut. P. 267.

Kamal, M., Watabe, S. & Hashimoto, K. 1991. Post-mortem changes in myofibrilla ATPase of sardine ordinary and dark muscles. Bull. Jpn. Soc. Sci. Fish. 57, pp. 1177 - 1184.

Kanoh, S., Suzuki, T., Mayeyama, K., Takewa, T., Watabe, S. & Hashimoto K. 1986. Comparative studies on ordinary and dark muscle of tuna fish. Bull. Jpn. Soc. Sci. Fish. 52, pp. 1807 - 1816.

Karube, I., Matsuoka H., Suzuki S., Watanabe, E., & Toyama, K. 1984. Determination of fish freshness with an enzyme sensor system. J. Agric. Food Chem. 32, pp. 314 - 319.

Kemp, B. & Spinelli, J. 1969. Comparative rates of IMP degradation in unfrozen and frozen-and thawed (slacked) fish. J. Food Sci., 34, pp. 133 - 135.

Kennish, J.M. & Kramer, D.E. 1987. A Review of High-Pressure Liquid Chromatographic Methods for Measuring Nucleotide Degradation in Fish Muscle. *In*: Kramer, D.E. & Liston, J.C. (eds.). Seafood Quality Determination. Elsevier Science Publisher B.V. Netherlands. Pp. 209 - 220.

Kiesvaara, M., Heiniö, R.-L., Mustranta, A. Hattula, T., Vartiainen, T. & Hallikainen, A. 1992. Silakka ravintona (Baltic herring as food). Helsinki: Elintarvikeviraston julkaisuja 11/1992. 14 p. (In Finnish.)

Konosu, S. & Yamaguchi, K. 1982. The flavour components in fish and shellfish. *In*: Martin, R.E, Flick, G.J., Hebard, C.E. & Ward, D.R. (Eds.). Chemistry & Biochemistry of Marine Food Products. Avi Publishing Company Inc, Westport CT, USA. Pp. 367 - 404.

Korhonen, R.W., Lanier, T.C. & Giesbrecht, F. 1990. An evaluation of simple methods for following rigor development in fish. J. Food Sci., 55, pp. 346 - 348, 368.

Kunimoto, M., Kochi, M., Moritsugu, H., Hatate, H., Kaneniwa, M., Ishizaki, S., Fujimoto, M. & Nakamura, M. 1993. Changes in K value and taste potentiating components in the kipper of round erring, *Etrumeus teres*, during Drying. J. Shimonoseki Univ. Fish. 41(4), pp. 229 - 235.

Kuninaka, A., Kibi, M. & Kin'ichiro, K. 1964. History & development of flavor nucleotides. Food Technol., 18(3), pp. 287 - 293.

Laird, W.M., Mackie, I.M. & Hattula, T. 1980. Studies of the changes in the proteins of cod-frame minces during frozen storage at -15 ^{o}C. *In*: Connell, J.J. (ed.). Advances in Fish Science and Technology. Fishing News Books Ltd, Farnham, Surrey, England. Pp. 428 - 434.

Love, R.M. 1974. Towards a valid sampling technique. *In*: Love, R.M. (ed.). The Chemical Biology of Fishes. Academic Press, London and New York. Pp. 1 - 59.

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

Luten, J.B., Brunner, K.K., Riekwel-Booy, G., Burggraaf, M.M. & Seuren, L.A.J. 1988. Kwaliteitaspecten van tong. Voedingsmiddeelentechnologie 21(1), pp. 28 - 32.

Mackie, I. 1980. A review of some recent applications of electrophoresis and iso-electric focusing in the identification of species of fish in fish and fish products. *In*: Connell, J.J. (ed.). Advances in Fish Science and Technology. Fishing News Books Ltd, Farnham, Surrey, England. Pp. 444 - 451.

Mackie, I.M. 1993. The effects of freezing on flesh proteins. Food Rev. Int. 9(4), pp. 575 - 610.

Makarewicz, W. 1969. AMP-aminohydrolase in muscle of elasmobranch fish. Purification procedure and properties of purified enzyme. Comp. Biochem. Physiol. 29, pp. 1 - 26.

Makarewicz, W. & Zydowo, M. 1962. Comparative studies on some ammonia producing enzymes in the excretory organs of vertebrates. Comp. Biochem. Physiol. 6, pp. 269 - 275.

Malle, P. & le Pezennec, I. 1992. Rapid determination of fish freshness by evaluation of ATP degradation reflected in K value. Sci. Aliments, 12, pp. 257 - 269.

Marseno, S.W., Hori, K. & Miyazawa, K. 1992. Distribution of 5'.nucleotidase in muscle of some marine fishes. Comp. Biochem. Physiol. 102B, pp. 247 - 253.

Marseno, D.W., Hori, K. & Miyazawa, K. 1993a. Purification and properties of membrane-bound 5'-nucleotidase from black rockfish (*Sebastes inermis*) muscle. J. Agric. Food Chem. 41, pp. 863 -869.

Marseno, D.W., Hori, K. & Miyazawa, K. 1993b. Purification and properties of cytosol 5'-nucleotidase from black rockfish (*Sebastes inermis*) muscle. J. Agric. Food Chem. 41, pp. 1208 - 1212.

Murata, M. & Sakaguchi, M. 1989. The effects of phosphatase treatment of yellowtale muscle extracts and subsequent addition of IMP on flavour intensity. Nippon Suisan Gakkaishi 55(9), pp. 1599 - 1603.

Nambudiri, D.D. & Gopakumar, K. 1992. ATPase and lactate dehydrogenase activities in frozen stored fish muscle as indices of cold storage deterioration. J. Food Sci. 57, pp. 72 -76.

Nedachi, K. & Hirota, N. 1992. Purification and properties of 5'-nucleotidase from snapper muscle. Nippon Suisan Gakkaishi 58(19), pp. 1905 - 1911.

Negishi, S, & Karube, I. 1989. An enzymatic assay method for IMP determination using IMP Dehydrogenase and an application of principle to a test paper method. Nippon Suisan Gakkaishi, 55(9), pp. 1591 - 1597.

Nguyen, A.L., Luong, J.H.T. & Masson, C. 1990. Determination of nucleotides in fish tissues using capillary electrophoresis. Anal. Chem. 62, pp. 2490 - 2493.

Nielsen, J. 1995. Sensory changes. In Huss, H.H. (ed.). Quality and Quality Changes in Fresh Fish. FAO Fisheries Technical Paper. No. 348. Rome, FAO. Pp. 35 -39.

Nikkilä, O.E. 1951. Formation of trimethylamine in the spoilage of fish. Suomen Kemistilehti 24, pp. 54 - 60.

Oba, K. & Niwa, E. 1992. Suppressive effects of salts on degradation of ATP-related compounds in fish flesh during cold storage. J. Jpn. Soc. Food Sci. Technol. [Nippon Shokuhin Kogyo Gakkaishi], 39, pp. 1007 - 1010.

Oba, K. & Niwa, E. 1993. The mode of inhibition by salts to two enzymes involved in IMP degradation in fish flesh. Nippon Shokuhin Kogyo Gakkaishi, 40, pp. 583 - 588.

Oka, H. 1989. Packaging for freshness and the prevention of discoloration of fish fillets. Packag. Technol. Sci. 2, pp. 201 - 213.

Parkin, K.L. & Brown, W.D. 1982. Preservation of seafood with modified atmosphere. *In*: Martin, R.E. Flick, D.J., Hebard, C.E. & Ward, D.R. (eds.). Chemistry & Biochemistry of Marine Food Products. Avi Publishing Company, Westport, CT, USA. Pp. 453 - 465.

Pearson, D. 1970. Flesh food - meat and fish. *In*: The Chemical Analysis of Food. J & A Churchill, London. Pp. 396 - 397.

Ponce de Leon, S., Inoue, N. & Shinano, H. 1994. Shelf life of sardine and bacterial flora in brine containing acetic acid during immersed storage. Fish. Sci. 60, pp. 429 - 433.

Raffin, J.P. & Leray, C. 1980. Comparative study on AMP deaminase EC-3.5.4.6 in gill, muscle and blood of fish. Comp. Biochem. Physiol. 67B, pp. 533 - 540.

Raja, K.C.M. & Moorjani, M.N. 1971. Review: Nucleotides - A study of their degradation in fish under various conditions and its impact on flavour. J. Food Technol. 8(1), pp. 3 - 6.

Ryder, J.M. 1985. Determination of adenosinetriphosphate and its breakdown products in fish muscle by high-performance liquid chromatography. J. Agric. Food Chem. 33, pp. 678 - 680.

Sakaguchi, M. & Koike, A. 1992. Freshness assessment of fish fillets using the Torrymeter and K value. *In*: Huss, H.H., Jakobsen, M. & Liston, J.C. (eds.). Quality Assurance in the Fish Industry. Elsevier Science Publishers B.V., Amsterdam. Pp. 333 - 338.

Saito, T., Arai, K. & Matsuyoshi, M. 1959a. A new method for estimating the freshness of fish. Bull. Jpn. Soc. Sci. Fish. 24, pp. 749 - 750.

Saito, T., Arai, K. & Yajima, T. 1959b. Studies on the organic phosphates in muscle of aquatic animals - VII. Changes in purine nucleotides of red lateral muscle of fish. Bull. Jpn. Soc. Sci. Fish. 25, pp. 573 - 575.

Shen, L., Yang, L. & Peng, T. 1996. Amperometric Determination of fish freshness by a hypoxanthine biosensor. J. Sci. Food Agric. 70, pp. 298 - 302.

Sivertsvik, M., Rosnes, J.T., Vorre, A., Randell, K., Ahvenainen, R. & Bergslien, H. 1996. Chilled transport of bulk packaged salmon (*Salmo salar*) in modified atmosphere. NORCONSERV Report no. 6-1966. Stavanger, Norway. 13 p. + app. 6 p.

Sjöblom, V. 1961. Silakka. *In*: H. Pitkänen (ed.). Suuri Kalakirja (Great Fish Book). Kustannusyhtiö Otava, Helsinki. Pp. 38 - 68. (In Finnish.)

Sorensen, N.K. 1992. Physical and instrumental methods for assessing seafood quality. *In*: Huss, H.H., Jakobsen, M. & Liston, J.C. (eds.). Quality Assurance in the Fish Industry. Elsevier Science Publisher B.V., Amsterdam. Pp. 321 - 332.

Spencer, R. & Baines, C.R. 1964. The effect of temperature on the spoilage of wet white fish. 1. Storage at constant temperatures between -1^{o} and 25^{o}C. Food Technol., Vol. 18, May 1964, pp. 769 - 773 (175 - 179).

Spinelli, J. & Miyauchi, D. 1968. The effect of 5' inosine monophosphate on the flavor of irradiated fish fillets. Food Technol. 22 (June), pp. 781 -783.

Surette, M.E. 1987. Isolation and Immobilization of Nucleoside Catabolic Enzymes for Evaluation of Fish Freshness. PhD. Thesis. Technical University of Nova Scotia, Halifax, NS, Canada. 82 p.

Tarr, H.L.A. 1955. Fish muscle riboside hydrolases. Biochem. J. 59, pp. 386 - 391.

Tarr, H.L.A. 1966. Post-mortem degradation of glucogen, nucleotides, sugar phosphates and sugar in fish muscle. A review. J. Food Sci., 31, pp. 846 - 854.

Thomson, A.B., McGill, A.S, Murray, J., Hardy, R. & Howgate, P.F. 1980. The analysis of a range of non-volatile constituents of cooked haddock (*Gadus aeglefinus*) and the influence of these on flavour. *In*: Connell, J.J. (ed.). Advances in Fish Science and Technology. Fishing News Books Ltd, Farnhm, Surrey, England. Pp. 484 - 488.

Tomioka, K. & Endo, K. 1984. Purification of 5'-nucleotidase from carp muscle. Bull. Jpn. Soc. Sci. Fish. 50, pp. 1077 - 1081.

Yokoyama, Y., Takahashi, S., Sakaguchi M., Kawai, F. & Kanamori, M. 1994. Post mortem changes of ATP and its related compounds and freshness indices in spear squid Doryteuthis bleekeri muscles. (Abstract in FSTA, Vol. 27 (5) 5 R 56). Fish. Sci. 60(5), pp. 589 - 587.

Yamamoto, H., Tomioka, K., Kawai, H. & Endo, K. 1986. Purification and properties of 5'-nucleotidase from cod muscle. Agric. Biol. Chem. 50, pp. 1123 - 1129.

Whittle, K.J., Hardy, R & Hobbs, G. 1990. Chilled fish and fishery products. *In:* Chilled Foods, the State of Art, Edited by T.R. Gormley. Elsevier Applied Science, London & New York. Pp. 87 - 116.

Williams, D.J., Poole, S.E. & Petroff, M. 1991. Inosine and hypoxanthine formation in tropical fish and its use in fish species differentiation. Food Aust. 43(11), pp. 500 - 502

Williams, D.J., Nottingham, S.M., Poole, S.E. & Petroff, M. 1993. Nucleotide degradation profiles of iced barramundi (Lates calcarifer) and Nile perch (Lates niloticus) muscle. J. Aquat. Food Prod. Technol. 2, pp. 63 - 74.

Wong, N.P., Damico, J.N. & Salvin, H. 1967. Investigation of Volatile Compounds in Cod Fish by Gas Liquid Chromatography and Mass Spectrometry. J. Assoc. Off. Anal. Chem. 50(1), pp. 8 - 15.