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Studies on Keeping Quality and Freshness of the Marine Products

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Abstract In order to keep the quality and freshness of the fishery products, various treatments were studied on black sea bream (*Acanthopagrus schlegeli*) and shrimp (*Trachypenaeus curvirostris*) stored in ice and at 5°C, mackerel (*Scomber japonicus*) and sardine (*Sardinops melanosticta*) stored at 5°C, and oyster (*Crassostrea gigas*) in shucked and unshucked forms stored in ice and at 5°C.

In general, among the samples packed by usual polyethylene bags, samples treated by 0.3% of modified amino preservating agent (called Pichi-Pichi, PP) have lower K-value and higher IMP content. The ability of PP to suppress the increasing rate of K-value (IRK) and to restrain decreasing rate of IMP (DRI) was slightly higher than saturated ozone treatment, higher than chlorinated treatment (0.04 ppm) and control. Samples packed by laminated-Ag-zeolite polyethylene bags (Zeomic) have lower bacterial counts, IRK and DRI than usual one.

The world production of fish and shellfish totaled about 75 million metric tons per year and of this total approximately 11 million metric tons were produced in Japan, and the recent trend of sea food consumption in the world has been toward various fresh forms (KAYAMA, 1986). As well known, fishes and shells are so perishable that many attempts have been done in order to keep either quality or freshness of the fishery products.

Modified ice storage with salt and chemical treatments extended the shelf life of shrimp (HO *et al.*, 1986), fish treated by ozone once per two days lengthened for 1.2–1.6 times (HARAGUCHI *et al.*, 1969), ultra violet light treated mackerel wrapped in 1mm polyethylene and packed in ice −1°C had at least a 7 days longer shelf life than conventional ice-packed untreated control (HUANG and TOLEDO, 1982). Utilization of glucose oxydase on fish extended period of sensory acceptance and delayed onset of putrefactive odors 21 days vs 15 days for control

(FIELD *et al.*, 1986). Appllication of antibiotic aureomycin chlortetracycline for oyster was done (ABBEY *et al.*, 1957; NOVAK *et al.*, 1958), and also modified atmosphere storage of rockfish and silver salmon under CO_2 was effective in reducing the formation of trimethylamine and ammonia and markedly inhibited the microbial growth (BROWN *et al.*, 1980).

However, the previous works were done in the different condition and indices of quality, and also some of them used the complicated apparatus and high economic cost. The objective of this study was to compare the effect of various treatments on the keeping quality and freshness of marine products which were kept in same condition and measure by means of K-value (SAITO *et al.*, 1959) and IMP content (FATIMA *et al.*, 1981) as the indices of freshness and quality.

MATERIALS AND METHODS

Treatment Groups

Treatments were divided into 6 groups. Group I is chlorinated natural sea water (nsw), Cl. This solution was made by passing the nsw into the chlorinizer tank, up to about 0.04 ppm content of chlorine in nsw. Group II is untreated nsw as control, C. Group III is nsw saturated with ozone, O_3. Group IV is nsw treated by 0.3% of modified amino preservating agent (Tominaga, 1986), called Pichi-Pichi (TōyūCO., Ltd., PP). Group V (NP) and VI (SP) were same with group II except using Zeomic bag for the former. All of the solutions were used as dipping and filling solution, except for fish stored without filling solution.

Sample Preparation

Oyster (*Crassostrea gigas*), black sea bream (*Acanthopagrus schlegeli*), mackerel (*Scomber japonicus*), sardine (*Sardinops melanosticta*) and shrimp (*Trachypenaeus curvirostris*) were selected for this study. Oyster, black sea bream and shrimp were obtained from Yasuura and Kawajiri, Hiroshima Prefecture. Mackerel and sardine were obtained from the Sagami Bay. The samplings were done from Autumn to Winter season (October, 1987–February, 1988). Immediately after catching, samples were divided into the 6 groups, and treated with solution as described above by the following procedure.

Black sea bream were divided into the 6 groups and then immediately soaked into the each treated solution for 40 minutes, killed by nailing the head, packed by usual polyethylene bag (for Group I to IV), and Group V and VI were packed by other kind of polyethylene bag, which will be described later. All of the samples were packed without filling solution, one bag for one fish and air was removed as much as possible before sealing. First set of 6 groups were kept on ice storage, and the second was kept on 5°C. All samples packed in an ice box during transportation to the laboratory. Mackerel and sardine were done by the same procedure, then kept at 5°C, and were treated as Group II and IV.

Oyster were shucked carefully, and the whole bodies were soaked into the each treated solution for 20 min., and following procedure was same with black sea bream as above. Each polyethylene bag contains 100 g of whole bodies and 100 ml of filling solution.

Unshucked oyster samples were prepared by soaking the oyster together with shell into the solution of Group II and IV, for 20 min., then packed by usual polyethylene bag. Each pack contains of 6 unshuked oysters and 100 ml of filling solution. Samples were stored in ice storage and 5°C.

Fresh shrimps were soaked into the solution of Group I, III, IV, V and VI for 30 min., then packed by usual polyethylene bag (for Group I, III and IV) and other kind of

polyethylene bag (for Group V and VI).

Packaging of Samples

Samples of Group I, II, III and IV were packed by usual polyethypene bag, sample of Group V was packed by another polyethylene bag (called NP), and sample of Group VI was same kind with Group V, but inside of the pack was laminated by Ag-zeolite (SP).

Extraction Procedure

The extraction procedure of TSUCHIMOTO *et al.* (1985) and Ryder (1985) with slight modification was adopted. One gram of muscle was homogenized with 2 ml of 10% perchloric acid (PCA) at ice temperature with a glass homogenizer. The homogenate was centrifuged at 1,900 $\times$g (3,000 rpm) for 10 min. at 0°C, and the supernatant was kept in ice, and the precipitate was mixed with 2 ml of 5% cold PCA, and then centrifuged at 1,900 $\times$ g for 3 min. at 0°C. The resulted supernatant was collected and immediately neutralized to pH 6.4–6.5 with KOH solution. After standing at 0°C for 30 min., it was centrifuged at 1,900$\times$g for 3 min. The supernatant was kept in ice, and the precipitate was mixed with 2 ml of neutralized 5% cold PCA of pH 6.4, followed by centrifugation at 1,900$\times$g for 3 min. at 0°C. The resulted supernatant was collected and diluted to 10ml by neutralized 5% cold PCA of pH 6.4. All of the samples were stored at -40°C until the time of analysis of ATP related compounds by HPLC.

HPLC Analysis

The HPLC was performed according to the method of TSUCHIMOTO *et al.* (1985) with slight modification by a Shimadzu HPLC system (Kyoto, Japan). HPLC pump (Model LC–5A) equipped with an injector (Model SIL–IA), a program panel of gradient (Model GRE–2B), a unit panel of elution holder (Model GRE–2B), a chromatocorder (Model 11, System Instrument Corporation), a UV-detector (Model 655A–21, Hitachi). The column used was a reversed phase type ODS stainless steel column Cosmosil 5 C–18P (4.6 mm I.D $\times$ 15 cm, Nakarai Chemicals, Ltd.) connected with gel column type 10 C–18 (4.6 mm I.D $\times$ 5 cm, Nakarai Chemcals, Ltd.).

The operation condition of HPLC by the methods of TSUCHIMOTO *et al.* (1985) and SUWETJA (thesis) were slightly modified. Two eluents were used, namely, the one eluent A was 0.05 M potassium phosphate buffer of pH 6.78, and the other eluent B was 37.5% methanol in 0.05 M potassium phosphate buffer of pH 6.78. Both the eluents were used at flow rate 0.7ml/min., and operation pressure was about 100 kg/cm^2, and absorbance was at 254 nm. All of the solution and samples must be filtered by 0.45 μm of Toyo filter paper prior to injection into the column, and injected volume was 20 μl. The area of peaks were automatically calculated by the chromatocorder, and the quantity of ATP and related compounds were identified by comparing with the standards.

Microbiological Analysis

As the indicator of spoilage of the fish samples, aerobic plate count (35°C, 2 days with standard agar), colform bacterial count (35°C, 1 day with Desoxycholate Agar), and psychrophilic bacterial count (20°C, 10 days with standard agar) were enumerated.

Samely on the oyster samples, aerobic plate count (35°C, 1 day with standard agar), calculation of most probable number (MPN) of *E. coli* (44.5°C, 1 day with EC broth) and halophilic bacterial count (35°C, 1 day with 7% NaCl supplemented standard agar) were carried out. Not all of the samples were enumerated their bacterial counts.

Organoleptic Test

The sensory evaluation was stressed on appearance of whole body of the samples and smell of raw muscle. Scores were given by 6 to 8 panelist on a 9 point hedonic scale (9 = like extremely; 5 = neither like or dislike; 1 = dislike extremely). Data were calculated by method of the total score divided by the number of panelist.

RESULTS

Change of K-value during Ice Storage

Change patterns of K-value of black sea bream during ice storage are shown in Fig. 1. From the data we can calculate IRK, which is K-value at t_2 minus K-value at t_1 divided by storage time during t_2-t_1. IRK of black sea bream treated by O_3, PP, Cl, C, NP and SP were 2.03, 1.70, 2.30, 2.41, 1.75 and 2.88 %/day during day 3 to day 15 storage. Furthermore, IRK

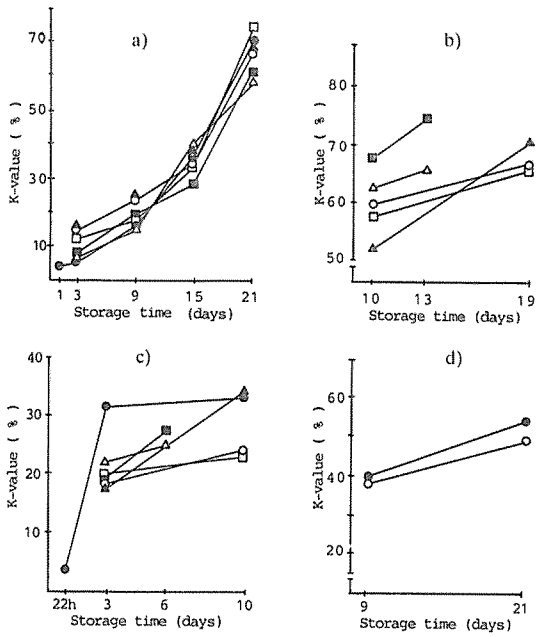

Fig. 1: Change of K-value of black sea bream (a), shrimp (b), shucked oyster (c), and unshucked oyster (d) by various treatments during ice storage.

▲—▲: Treatment by chlorine (Group I)
●—●: Control (Group II)
□—□: Treatment by ozone (Group III)
○—○: Treatment by modified amino preservating agent (Group IV)
■—■: Treatment by polyethylene bag (Group V)
△—△: Treatment by laminated Ag-zeolite polyethylene bag (Group VI)

changes from day 15 to day 21 were 6.62, 5.11, 4.96, 5.75, 5.30 and 2.86 %/day, respectively. However, IRKs during 21 days storage of those were 3.54, 3.15, 3.23, 3.29, 2.88 and 2.79 %/day, respectively. It was appeared that PP was still effective as an preservating agent and also NP and SP. O_3 was effective only up to 15 days storage. In this case Cl had only a little effect as an preservating agent. The chromatogram profiles of HPLC of black sea bream are shown in Fig. 7.

The K-values of shrimp during ice storage are shown in Fig. 1. PP and SP had a lower increasing pattern than O_3, NP and Cl. IRK values during day 10 to day 19 of PP, O_3 and Cl were 1.29, 1.70 and 4.00 %/day, respectively. Other samples packed by another polyethylene bag had IRK 0.95 and 3.09 %/day during day 10 to day 13, respectively, for SP and NP. Total of IRK of PP, O_3 and Cl during 19 days storage were 3.53, 3.52, and 3.73 %/day. SP and NP had IRK value 5.09 and 5.72 %/day.

Changes of K-value pattern of shucked oyster during ice storage are shown in Fig. 1. The K-value of C increased rapidly to 9.40 %/day, then slightly increased up to day 10. However, K-value of C and Cl were higher than PP and O_3. IRKs of PP, O_3, Cl and C were 2.50, 2.40, 3.45, 3.40 %/day, respectively, during 10 days storage. SP and NP had IRK-value 4.26 and 4.59 %/day during 6 days storage. The chromatogram profiles of HPLC of shucked oyster are shown in Fig. 7.

Changes of K-value of unshucked oyster treated by PP are shown in Fig. 1. IRKs of PP and C were 2.38 and 2.65 %/day during 21 days storage. However PP had an ability to suppress the increasing rate of K-value.

Change of K-value during 5°C Storage

Fig. 3 shows K-value at 8 days storage of black sea bream. PP, O_3, Cl, C, NP and SP were 40.0, 38.0, 40.5, 43.5, 33.0 and 31.5 % respectively. Their IRKs were 5.0, 4.75, 5.06, 5.44, 5.13 and 3.94 %/day, respectively.

IRK of mackerel treated by PP was lower than C as indicated by 8 %/day for PP and 8.5 %/day for C. Also from this data it appears that PP has an ability to suppress the increasing rate of K-value. Total of IRKs during 6 days storage of PP and C were 8.17 and 8.92 %/day, respectively.

Sardine treated by PP also gives a better result than C. At day 0 to day 4 the IRK of PP and C were 8.25 and 10.0 %/day.

IRKs of shrimp during 7 days storage for PP, O_3, Cl, NP and SP were 5.86, 8.64, 8.43, 7.21 and 8.43 %/day, respectively.

PP also can suppress the IRK-value of unshucked oyster, IRKs of PP and C during 9 days storage were 1.28 and 1.55 %/day.

Generally, according to the above data it was shown that, among the samples that packed by usual polyethylene bag, PP had an ability to suppress the IRK greater than Cl. IRK of PP was almost same with O_3 on black sea bream, but on shrimp PP has greater ability in suppressing of IRK-value than O_3.

Change of Inosine 5'-monophosphate (IMP) during Ice Storage

Fig. 2 shows the dephosphorilation of IMP during ice storage in % (g/100g of wet muscle). Black sea bream treated by PP and O_3 had lower DRI than C and Cl during 3 to 15 days storage, but from 21 days storage samples treated by O_3 were rapidly decreased in the content of IMP. DRIs from 0 to 21 days storage of PP, O_3, Cl, C, NP and SP were 34×10^{-4}, 13×10^{-4}, 31×10^{-4}, 29×10^{-4}, 48×10^{-4}, and 43×10^{-4} %/day, respectively.

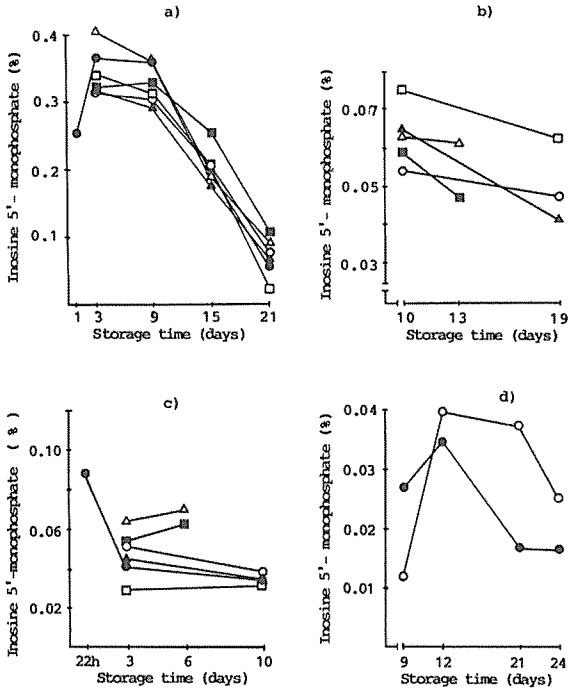

Fig. 2: Change of inosine 5'-monophosphate of black sea bream (a), shrimp (b), shucked oyster (c), and unshucked oyster (d) by various treatments during ice storage.
Symbols are same as Fig. 1.

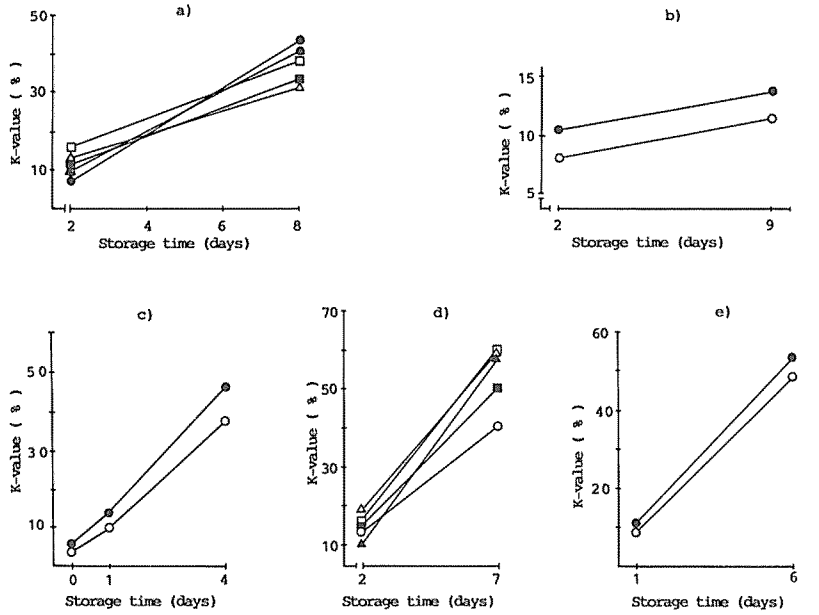

Fig. 3: Change of K-value of black sea bream (a), unshucked oyster (b), sardine (c), shrimp (d) and mackerel (e) by various treatments during storage at 5°C.
Symbols are same as Fig. 1.

On shrimp treated by PP, O_3, Cl, DRI values from 10 to 19 days storage were 6.67×10^{-4}, 13.33×10^{-4}, 25.56×10^{-4} %/day, respectively, so that PP had an ability to inhibit the dephosphorilation of IMP greater than other treatments. SP and NP showed DRI values of 6.67×10^{-4} and 40×10^{-4}, respectively, during 10 to 13 days storage. It was appear that SP was better than NP.

On shucked oyster, PP has higher content of IMP than others after 10 days storage. This fact also occurred on unshucked oyster treated by PP.

Change of Inosine 5'-monophosphate(IMP) during 5°C Storage

Fig. 4 shows the dephosphorilation of IMP during 5°C. DRIs of black sea bream treated by PP, O_3, Cl, C, NP and SP during 2 to 8 days storage were 0.013, 0.011, 0.023, 0.023, 0.016 and 0.140 %/day, respectively. On mackerel and sardine, PP could restrain the dephosphorilation of IMP better than C. The dephosphorilations of IMP of shrimp treated by PP, O_3, Cl, NP and SP during 2 to 7 days were 0.018, 0.021, 0.025, 0.002 and 0.018 %/day, respectively. This fact indicated that PP was able to defend the dephosphorilation of IMP better than O_3 and Cl. In this case the content of IMP of NP was higher than SP during 2 to 7 days storage.

Organoleptic Test

Means of organoleptic scores for raw muscle smell and appearance of whole body of the samples are summerized in Fig. 5. According to the panel test, smell of raw muscle (without skin) of black sea bream treated by PP, O_3 and SP still remained acceptable, and sample treated by Cl almost became unacceptable, whereas samples treated by NP and C were already

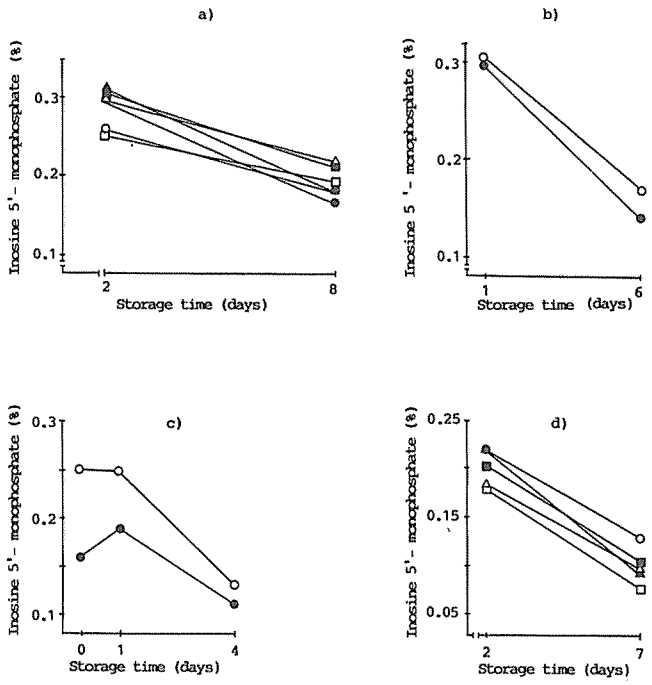

Fig. 4: Change of inosine 5'-monophosphate of black sea bream (a), mackerel (b), sardine (c), and shrimp (d) by various treatments during storage at 5°C.
Symbols are same as Fig. 1.

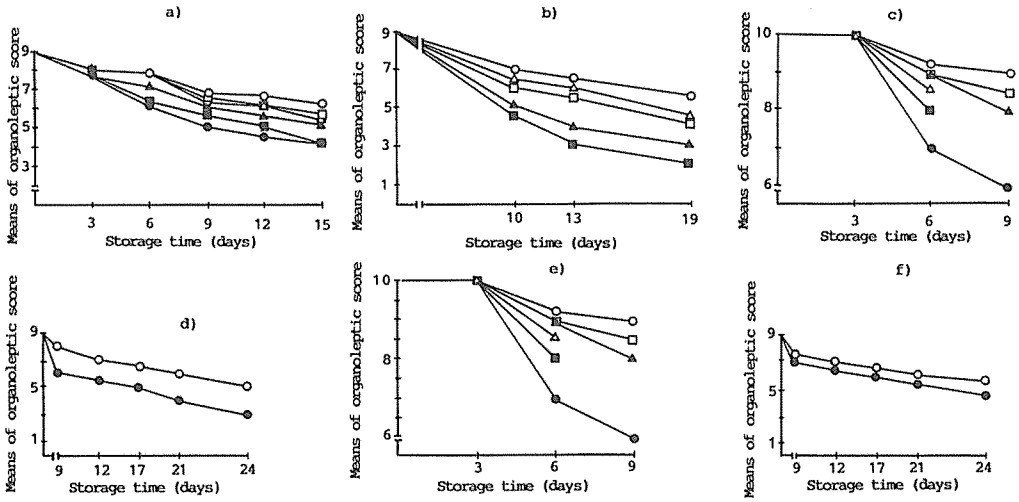

Fig. 5: Means of organoleptic scores of smell of raw muscle of black sea bream (a), shrimp (b), shucked oyster (c), and unshucked oyster (d) by various treatments during ice storage and means of appearance of shucked oyster (e) and unshucked oyster (f) muscle during ice storage.
Symbols are same as Fig. 1.

unaccepted at 15 days storage. NP and C might produce the unspecific raw muscle odor of fish due to bacterial spoilage, eventhough NP had high content of IMP. Until 19 days storage, shrimp treated by PP had the highest score, eventhough at that day PP was not the highest in content of IMP. This might mean that PP can produce the specific smell of marine food products. Shucked oyster treated by PP also gave the highest score until 9 days storage. Smell of raw muscle of unshucked oyster treated by PP still remained acceptable up to 24 days storage, while C was already unacceptable at 20 days storage.

Appearance of shucked oyster treated by PP which stored in ice up to day 9 gave the highest score, also on unshucked oyster treated by PP up to 24 days ice storage still remained

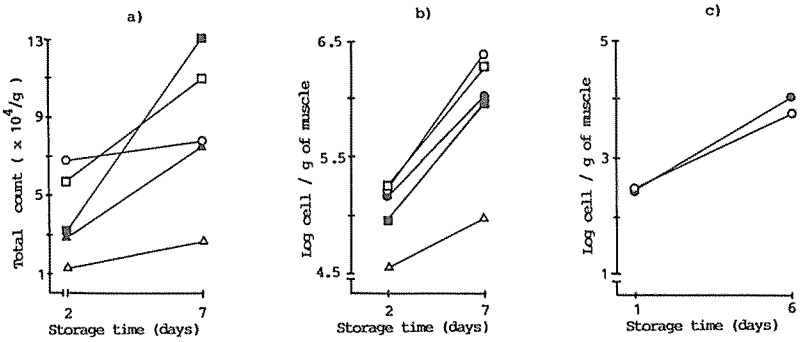

Fig. 6: Change of total bacterial counts (a) and psychrophilic bacteria (b) of shrimp and total bacterial counts of mackerel (c) by various treatments during storage at 5°C.
Symbols are same as Fig. 1.

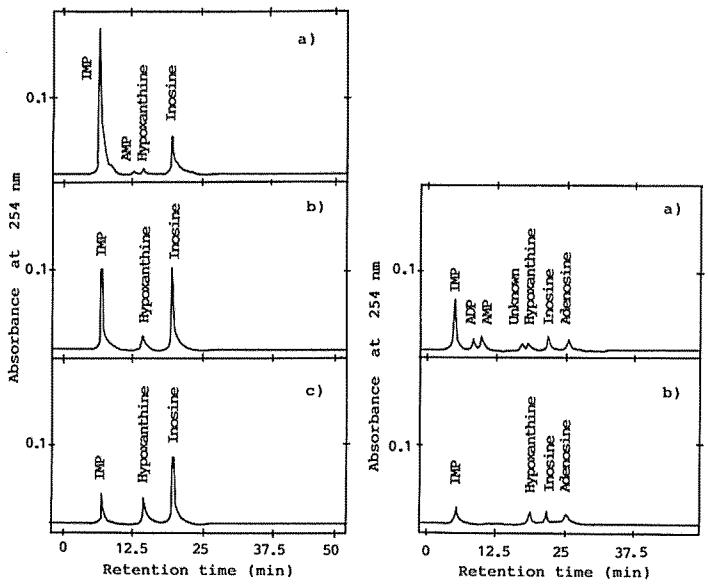

Fig. 7: Chromatogram profiles of ATP and its breakdown products in black sea bream (*Acanthopagrus schlegeli*, left) and shucked oyster (*Crassostrea gigas*, right). The fish were storaged in ice for 3 (a), 15 (b) and 21 (c) days, and the shucked oyster were storage in ice for 3 (a) and 10 (b) days after treating with 0.3% modified amino preservating agent (PP) solution.

acceptable, while C was already unacceptable.

Microbiological Analysis

Microbial examination on shrimp and mackerel were shown in Fig. 6. It appeared that SP had an ability to inhibit either the growth of total bacterial or psychrophilic bacterial counts on shrimp during storage at 5°C. In the case of mackerel treatment by PP had an inhibiting effect on growth of total bacterial counts greater than C.

DISCUSSION

According to the results described above, it appears that marine products treated by 0.3% modified amino preservating agent (called Pichi-Pichi; PP), in general, have a lower value in K-value and greater content in IMP than ozone and chlorine treated samples and control. PP has been developed for preservation of fishes caught by angler, but the mechanism of inhibition of PP in fish and shellfish muscle was still unclear. Data of this experiment suggest that PP has an ability to inhibit either ATP degradation or IMP dephosphorilation.

The data also show that ozone and chlorine are unstable during storage. On the black sea bream ozone had a good capability to suppress IRK up to 15 days in ice storage, then its ability as a preservating agent decreased rapidly. Many factors were affected on stability of ozone as a preservating agent especially in water as reported by other workers that organic loading and

poor ozone penetrability were key factors in effecting the ability of ozone to sterilize surface (RICKLOFF, 1987), moreover, the germicidal effects of ozone were affected by contact time, temperature, pH and presence of inorganic and organic materials in the solution (YANG and CHEN, 1979). In this experiment the saturated-ozone in natural sea water was used and treated with animals only at initial by dipping, while to preserve the saturated ozone solutions was difficult becuase of the great tendency of ozone to react or to undergo the decomposition (HANN and MANLEY, 1967). It was more clear that sodium chloride at a certain concentration accelerates the loss of ozone from solution (Rose and Kotula, 1984).

Chlorine treated samples also showed that chlorine with concentration 0.04 ppm in dipping and filling solution had only a little effect to suppress the IRK value and to restrain the dephosphorilation of IMP. It might be considered that the content of chlorine in dipping solution was too small, so that the preservating effect of chlorine was little. As described by KELLY *et al.* (1981) that reduction in bacterial number with chlorine on lamb carcases was affected by chlorine concentration. Also using of chlorine on fresh chicken meat for a dipp just prior to packing did not appear to be satisfactory mean for increasing the shelf-life of poultry meat appreciably (ZIEGLER and STADLEMAN, 1955).

However, chlorine and ozone have main effect on reducing the bacteria, degradation process from ATP to inosine (HxR) or adenosine (AdR) is autolytic and fast reaction, then that from HxR or AdR to hypoxanthine (Hx), xanthin and uric acid is autolytic, bacteriolytic and slow reaction (JONES, 1966), so that in the former reaction step (ATP to HxR or AdR), chlorine and ozone might have no effect.

In the case of samples which were packed in polyethylene bag laminated by Ag-zeolite (SP) have better results than NP on suppressing IRK, and SP restrained the IMP dephosphorilation, because Ag ion has an ability to kill bacteria on the surface of samples. These results indicated that kind of packing materials, including regidity and porosity, affected on degradation of ATP and related compounds. However, since the mechanism of keeping quality by modified amino preservating agent and laminated Ag polyethylene bag still remains unclear, further studies are requested. More this kind of experiment is going to be extended to fresh water fishes such as rainbow trout (*Salmo gairdneri*) and ayu (*Plecoglossus altivelis*).

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水産魚介類の品質および鮮度維持に関する研究

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戦後食糧事情の厳しかった時代は魚介類の鮮度判定および保鮮技術に関する研究が多く行われた。その後コールドチェーンの一応の確立によって防腐問題の解決が図られ，鮮度問題は解決されたかにみなされてきたが，一方で品質保持に関して種々な改良がなされ，急速・極低温冷凍技術が発達するとともに，氷温貯蔵技術の進歩がもたらされ，鮮度判定法としても従来のVBN判定からATP関連物質判定へと発展してきた。しかし今日200海里時代に入り，わが国の漁業が著しい制約を受けるに及んで，水産資源の有限性に対する認識が昂まるとともに，水産資源の有効利用技術の開発が社会的に要請されるようになった。そのため，近時登場した鮮度保持剤を用いた沖締技術および保鮮包装剤について，それらの効果を検討しようとする研究を実施したので，報告する。

試験魚介類として，カキ，エビ，イワシ，サバ，タイ等を用い，天然海水を対照として塩素およびオゾン処理海水，ピチピチ（トーユー）添加海水（PP），それらとともにゼオミック（シナネンニューセラ）袋（SP）による保鮮効果を細菌数（一般細菌数，大腸菌数，低温細菌数），K値，およびIMP濃度，さらにパネルテストによって判定を行った。

クロダイを氷蔵した場合，15日迄の官能検査でPPが最高であり，5℃で貯蔵したものではK値およびIMPともSPおよびO_3が良く，イワシ，サバではK値およびIMPともPPは対照より優れていた。カキでは氷蔵下で9日迄官能検査で，殻付きでもPPは対照に勝り，K値からもPPは対照よりも良い結果が得られた。エビではPPがK値，IMPとも最高であり，一般細菌数，低温細菌数からみるとSPが最も優れていた。また官能検査での臭気からPPが最も良い結果を得た。