

Effects of Various Salts on IMPase Activity and IMP Quantity in Coho Salmon, *Oncorhynchus kisutch* (Walbaum 1792), Salmonidae

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Abstract

Coho salmon, *Oncorhynchus kisutch* (Walbaum 1792), Salmonidae is a fast growing species that is reared for food worldwide. After harvest, coho salmon is often salted and stored. When a fish dies, adenosine triphosphate (ATP) is degraded in the muscles to inosinic acid (IMP), the taste component that gives the fish its flavour. During storage, IMP is degraded to non-taste components by the IMP-degrading enzyme (IMPase), resulting in a loss of flavour. Therefore, it is crucial to suppress IMPase activity in order to maintain IMP levels. In this study, IMPase activity was investigated at various pH levels and with salts used for storage of coho salmon, along with the resulting amount of IMP and changes in pH level. The results showed that the amount of IMP significantly decreased from $9.5 \mu\text{mol}\cdot\text{g}^{-1}$ on day 0 to $4.6 \mu\text{mol}\cdot\text{g}^{-1}$ on day 4 ($p < 0.05$) in fish stored at 4°C in our laboratory but the pH level remained constant at 6.1 ± 0.1 . The enzyme activity was the highest at pH 6 and decreased sharply at pH 7-8. The salts that effectively maintained IMP in coho salmon were in the following order, MgSO_4 and Na_2SO_4 at pH 4-5; MgCl_2 , CaCl_2 , MgSO_4 , and Na_2SO_4 at pH 6; and NaCl , CaCl_2 , and Na_2SO_4 at pH 7-8. These results indicated that the amount of IMP present after several days of storage was significantly higher with the addition of these salts.

Introduction

Coho salmon, *Oncorhynchus kisutch* (Walbaum 1792) is one of the most important aquaculture species in the world, with the third largest production of approximately 137,500 tonnes per year after Atlantic salmon (1,426,000 tonnes per year) and rainbow trout (728,400 tonnes per year) (Japan Aquatic Oil Association 2012).

Coho salmon is a fast growing species that adapts well to the aquaculture environments. The fish is mainly farmed in Chile, Japan, the United States, Canada, and France, and is consumed

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worldwide (Nara 1992; Japan Aquatic Oil Association 2012; Koseki 2013; Nagasawa 2013). Fresh coho salmon is often eaten as a dish known as meunière; however, if it is stored, maintenance of the taste component inosinic acid (IMP) becomes a great concern.

Inosinic acid is a decomposition product of adenosine triphosphate (ATP) in fish, and an enzyme breaks down ATP as follows:

Adenosine triphosphate (ATP) → adenosine diphosphate → adenosinemonophosphate → inosinic acid (IMP) → inosine (HxR) → hypoxanthine.

Since ATP is degraded relatively earlier than IMP (Srirangsan et al. 2010), the latter accumulates in fish muscles. The enzyme IMPase degrades IMP slowly, hence, suppressing the activity of this enzyme can preserve IMP, and the flavour of the fish.

Considering that coho salmon is often stored by salting, understanding the effect of salt on IMPase activity is important. Various salts have an effect in suppressing IMPase activity in walleye Pollock, *Theragra chalcogramma* (Pallas 1814) and silver whiting, *Sillago japonica* Temminck and Schlegel 1843. There are several types of IMPase, and they function differently in different fish species (Tomioka and Endo 1984). Studies on IMPase in coho salmon or the effect of different salts on IMPase activity are lacking. Furthermore, because the enzyme activity is strongly affected by pH, elucidating the changes in its activity at various pH levels is necessary.

In this study, we investigated the sequential changes in IMP level and pH in coho salmon after treating with the main salt types used for storing salmon, namely NaCl, MgCl₂, CaCl₂, MgSO₄, and Na₂SO₄ (Niino et al. 2003) on the activity of IMPase at pH 4–8. This pH range is often used when fish flesh is preserved (Tomioka et al. 1986; Oka Foods Co., Ltd. 2012). We hypothesised that different salts would affect differently on the amount of IMP accumulated in coho salmon after several days.

Materials and Methods

Sample preparation

Coho salmon (each about 50cm in length), were obtained from an aquaculture farm at Ishinomaki, Miyagi, Japan. The fish were killed by pithing, and transported to the laboratory within 24 hours, in a frozen condition (0 °C; on ice in a cool box). In the laboratory (day 0), the fish samples were stored in an isothermal room at 4 °C. Three coho salmon were used for this experiment. Samples were analysed on days 0, 1, 2, 3, and 4. Reagent-grade chemicals were used in the experiment.

Measurement of IMP in coho salmon

Inosinic acid was extracted from 2 g muscle of the fish flesh by using 5 mL 10% perchloric acid (n=9; 3 muscle samples from 3 fish). The fish flesh was precipitated by centrifugation at 11,000 x g for 10 min at 5 °C, and 25 mL of supernatant was obtained. The supernatant was neutralised in KOH, and the neutralisation salt was precipitated by centrifugation at 12,000 x g for 5 min at 5 °C. Pure water prepared by filtering distilled water using an AUTOPURE WT100 purification system (Yamato Scientific Co., Ltd.) was then added to obtain 10 mL of supernatant. The supernatant was filtered using a Millipore filter (Millex-LG; 0.20 µm), and the amount of IMP was measured using high-performance liquid chromatography (analytical instrument: Hitachi L2130, column: Shodex GS-320 HQ, solvent: 200 mM NaH₂PO₄·2H₂O, flow rate: 0.6 mL·min⁻¹, temperature: 30 °C, detector: Hitachi L7420, and wavelength: 260 nm). The amount of IMP was measured daily for 4 days from day 0. The samples were stored at 4 °C for use on the following day.

Measurement of pH in coho salmon

The flesh of three fish was pricked with a pH electrode meant for food samples to measure the pH (Horiba Co., Ltd.). The pH was measured immediately posthumously on day 0 and daily for 4 days thereafter.

Assessment of IMPase activity and reaction time

The relationship between IMPase activity and reaction time was investigated. The IMPase was extracted from the fish samples as follows. Dorsal meat from three coho salmon was homogenised in three volumes at a ratio of 1:3 g of meat and water, and minced using a homogeniser. The homogenate was dialysed against water for 2 days, after which the dialysate was filtered using filter paper No. 1 (Advantec Co., Ltd.) and diluted twice at 10 °C (enzyme solution). Three standard mixtures were prepared and the standard reaction mixture consisted of 2.25 mL of buffer (50 mM maleic acid/Tris/NaOH [Perrin and Dempsey 1981]; pH 6), 0.25 mL of 25 mM IMP, and 0.5 mL of enzyme solution to a total volume of 4 mL. The reaction mixture was incubated at 25 °C for 0-48 h, and the reaction was stopped at regular intervals (3 h, 15 h, 20 h, 24 h, 39 h, 48 h) by adding 2 mL of 10% perchloric acid. The precipitate was separated by centrifugation, and the level of free inorganic phosphate was determined using the molybdenum blue method (Salt Industry Centre of Japan 2007). The amount of inorganic phosphate was considered to reflect the level of enzyme activity. Since the IMPase activity is measured when the enzymatic reaction speed is constant, we confirmed that IMPase activity and reaction time had a linear relationship.

Association of IMPase activity with pH in coho salmon

The IMPase extraction method was performed as described in the previous paragraph. The IMPase activity was measured using the standard reaction mixture, which consisted of 2.25 mL of buffer [50 mM succinic acid/NaOH at pH 4–6, and 50 mM maleic acid/Tris/NaOH at pH 6–8 (Perrin and Dempsey 1981)], 0.25 mL of 25 mM IMP, and 0.5 mL of enzyme solution in a total volume of 4 mL. The effective pH buffering ranges of these buffers were 3.8–6.0 and 5.2–8.6, respectively. The three standard mixtures described earlier were incubated at 25 °C for 24 h, after which the reaction was stopped by adding 2 mL of 10% perchloric acid.

The precipitate was separated by centrifugation, and the level of free inorganic phosphate was determined using the molybdenum blue method (Salt Industry Centre of Japan 2007). The relative IMPase activity was calculated assuming that the highest activity level was 100% activity.

Effects of various salts on IMPase in coho salmon

IMP-degrading enzyme was extracted as described above. The IMPase activity was measured by creating the standard reaction mixture, which consisted of; 2.25 mL of buffer (as described above) at pH 4–8, 0.25 mL of 25 mM IMP, 0.29 M NaCl, 0.18 M MgCl₂, 0.15 M CaCl₂, 0.14 M MgSO₄ or 0.12 M Na₂SO₄, and 0.5 mL of enzyme solution to a total volume of 4 mL. After the reaction mixture was incubated at 25 °C for 24 h, the reaction was stopped by adding 2 mL of 10% perchloric acid. The free inorganic phosphate level was measured as described above.

Quantification of IMP after adding various salts

After the various salts added, the amounts of IMP present were calculated as follows:

$$Day1_{IMP(recal)} = Day0_{IMP^{**}} - \{Salt_{effect} * (Day0_{IMP^{**}} - Day1_{IMP^{**}})\}$$

$$Day2_{IMP(recal)} = Day1_{IMP(recal)} - \{Salt_{effect} * (Day1_{IMP^{**}} - Day2_{IMP^{**}})\}$$

$$Day3_{IMP(recal)} = Day2_{IMP(recal)} - \{Salt_{effect} * (Day2_{IMP^{**}} - Day3_{IMP^{**}})\}$$

$$Day4_{IMP(recal)} = Day3_{IMP(recal)} - \{Salt_{effect} * (Day3_{IMP^{**}} - Day4_{IMP^{**}})\}$$

*Salt_{effect} = (50 mM succinic acid + 50 mM maleic acid) / 2 × 1 / 100 (at pH 6 for effect of various salts on the activity of IMPase)

**Day0_{IMP}– Day4_{IMP}(mean values of IMP)

Statistical analysis

Data for pH values and the variation for IMP per day, as well as IMPase activity variation per day for each salt and each pH value, were subjected to one-way analysis of variance by using the least significant difference method ($p < 0.05$). The daily mean values of IMP in the absence of salts and the amounts of IMP after addition of various salts were compared using a t -test at a significance level of $p < 0.05$.

Results

Amount of IMP and pH level in coho salmon stored at 4 °C

The changes in the amount of IMP in coho salmon stored at 4 °C, over the 4 days period are shown in Table 1. The average amount of IMP was $9.5 \mu\text{mol}\cdot\text{g}^{-1}$ on day 0, and it significantly decreased to $4.6 \mu\text{mol}\cdot\text{g}^{-1}$ on day 4 ($p < 0.05$). There was no increase in the IMP amount in this study, indicating that all ATP had degraded on day 0. The pH remained at 6.1 ± 0.1 from day 0 to 4 ($p > 0.05$).

Table 1. Mean values of inosinic acid (IMP) content and pH in coho salmon.

Day	IMP ($\mu\text{mol}\cdot\text{g}^{-1}$)	pH
0	9.5 ± 0.86	6.1 ± 0.03
1	8.0 ± 0.69	6.1 ± 0.04
2	7.2 ± 1.3	6.1 ± 0.06
3	7.3 ± 0.37	6.2 ± 0.09
4	4.6 ± 2.2	6.1 ± 0.16

Mean values of IMP ($n = 9$) differed significantly ($p < 0.05$) across each day.

Mean values of pH ($n = 3$) did not differ significantly ($p > 0.05$) across each day.

IMPase activity and reaction time

The relationship between IMPase activity and reaction time in coho salmon is shown in Fig. 1. There was a good correlation until 24 h. However, after 24 h, the enzymatic activity suddenly increased, and the correlation between enzyme activity and reaction time became weaker.

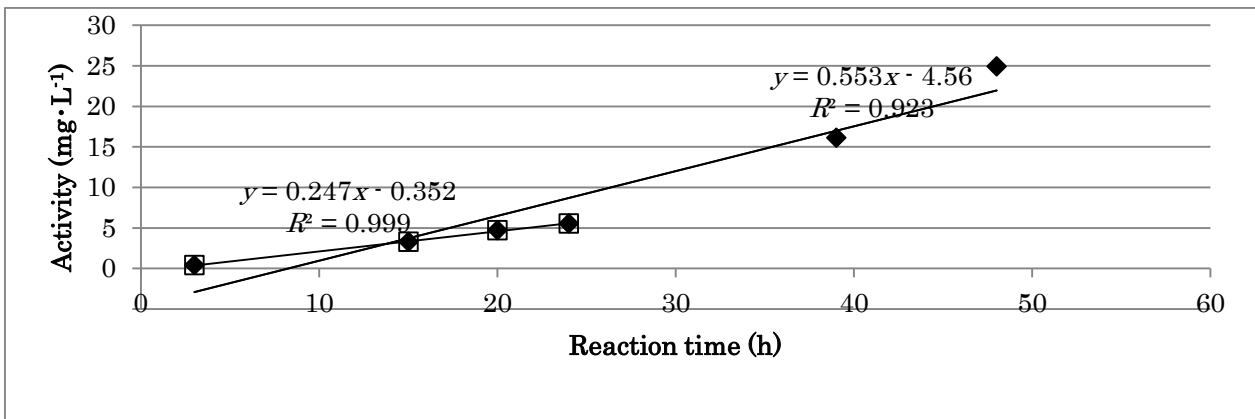


Fig. 1. Changes in IMPase activity in coho salmon samples versus reaction time.

◆ linear approximation: $y = 0.553x - 4.56$, coefficient of correlation: $R^2 = 0.923$; linear approximate equation for IMPase activity from 0 to 48 h. □ linear approximation: $y = 0.247x - 0.352$, coefficient of correlation: $R^2 = 0.999$; linear approximate equation for IMPase activity from 0 to 24 h.

Relationship between IMPase activity and pH

The IMPase activity increased to approximately 70-100% at pH 4-6 and was the highest at pH 6 (Fig. 2). The activity significantly decreased to approximately 10-40% at pH 6-8 ($p < 0.05$).

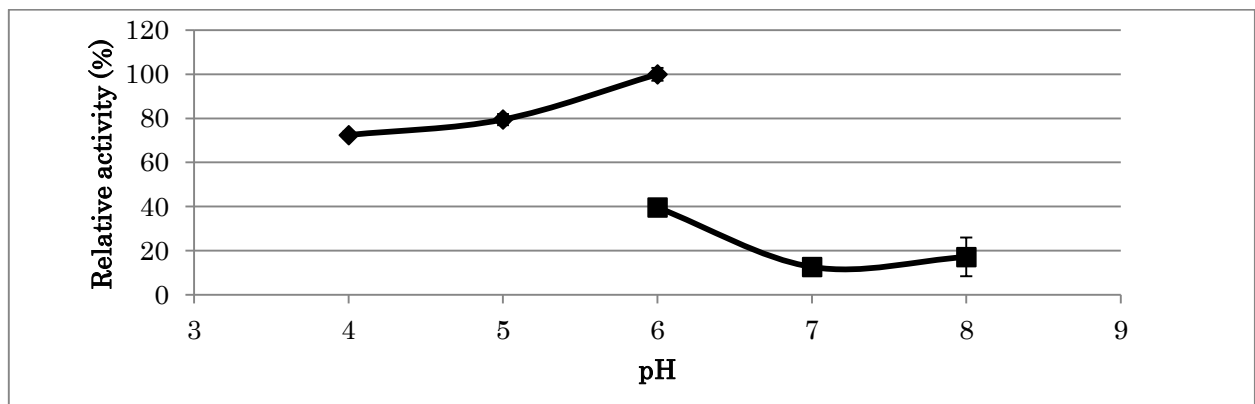


Fig. 2. Effect of pH on IMPase activity in coho salmon; bars denote standard deviation of the mean ($n = 3$). Mean values of relative activity indicate a significant ($p < 0.05$) difference for each pH. ◆ 50 mM succinic acid/ NaOH; ■ 50 mM maleic acid/ Tris/ NaOH.

Effect of various salts on IMPase activity

The IMPase activity at various pH levels for different salts is shown in Table 2. The IMPase activity decreased to approximately 80%, 44-51%, and 0-5% in the presence of NaCl, MgCl₂ and CaCl₂, and MgSO₄ and Na₂SO₄, respectively, at pH 4 ($p < 0.05$) and to 83%, 57-61%, and 17-19% in the presence of NaCl, MgCl₂ and CaCl₂, and MgSO₄ and Na₂SO₄, respectively, at pH 5 ($p < 0.05$).

Different salts had significantly different effects on IMPase activity, and the effects of MgSO_4 and Na_2SO_4 were particularly strong. The IMPase activity was affected by buffers and various salts used when the pH was maintained constant. At pH 6, the activity level was 62-93% when NaCl was added, 38-68% for MgCl_2 , 45-71%, for CaCl_2 , 57-58% for MgSO_4 , and 40-52%, for Na_2SO_4 ($p < 0.05$). The IMPase activity was reduced to approximately 10%, 34%, 36%, and 52% in the presence of CaCl_2 , NaCl, Na_2SO_4 , and MgCl_2 , respectively. Activity was remarkably increased by the addition of MgSO_4 (217%) at pH 7 ($p < 0.05$), and to 11%, 14%, 23%, and 46% in the presence of CaCl_2 , Na_2SO_4 , NaCl, and MgCl_2 , respectively, but was remarkably increased by the addition of MgSO_4 (144%) at pH 8 ($p < 0.05$). The IMPase activity was sharply reduced by the addition of CaCl_2 but remarkably increased by the addition of MgSO_4 .

Calculation of the amounts of IMP after adding various salts

The mean value of IMP without addition of salts and the calculation of the amounts of IMP by adding various salts on each day were compared using the *t*-test. The amount of IMP was 8.0-8.8 $\mu\text{mol}\cdot\text{L}^{-1}$ ($p > 0.05$) on day 1, 7.2-8.5 $\mu\text{mol}\cdot\text{L}^{-1}$ ($p < 0.05$) on day 2, 7.3-8.5 $\mu\text{mol}\cdot\text{L}^{-1}$ ($p < 0.05$) on day 3, and 4.6-7.3 $\mu\text{mol}\cdot\text{L}^{-1}$ ($p < 0.05$) on day 4. The addition of various salts after day 2 had different effects. The amount of IMP was determined in the presence and absence of various salts across different days (Table 3).

Table 2. Effect of various salts on the activity of IMPase in coho salmon.

Salt added	Mean values of relative activity (%)					
	pH 4 ^a	pH 5 ^a	pH 6 ^a	pH 6 ^b	pH 7 ^b	pH 8 ^b
None	100	100	100	100	100	100
NaCl	80±0.56	83±1.2	62±2.9	93±1.1	34±1.8	23±1.0
MgCl_2	44±0.84	57±0.90	38±0.26	68±2.1	52±2.9	46±2.4
CaCl_2	51±2.3	61±3.6	45±2.5	71±7.0	10±3.6	11±2.3
MgSO_4	0±0.43	17±0.65	58±0.44	57±2.1	217±39	144±51
Na_2SO_4	5±1.8	19±0.00	52±0.72	40±1.6	36±6.0	14±0.42

NaCl 0.29 M, MgCl_2 0.18 M, CaCl_2 0.15 M, MgSO_4 0.14 M, Na_2SO_4 0.12 M, $n = 3$. Mean values of relative activity were significantly different ($p < 0.05$) for the different salts at each pH value.

^a50 m M succinic acid/ NaOH.

^b50mM maleic acid/ Tris/ NaOH. The measured enzyme activity at 100% was 13.8 $\text{PO}_4 \text{ mg}\cdot\text{L}^{-1}\cdot 24 \text{ h}^{-1}$ at pH 4, 15.1 $\text{PO}_4 \text{ mg}\cdot\text{L}^{-1}\cdot 24 \text{ h}^{-1}$ at pH 5, 19.1 $\text{PO}_4 \text{ mg}\cdot\text{L}^{-1}\cdot 24 \text{ h}^{-1}$ at pH 6 (0.2 M succinic acid/NaOH), 7.5 $\text{PO}_4 \text{ mg}\cdot\text{L}^{-1}\cdot 24 \text{ h}^{-1}$ at pH 6 (50 mM maleic acid/ Tris/NaOH), 2.4 $\text{PO}_4 \text{ mg}\cdot\text{L}^{-1}\cdot 24 \text{ h}^{-1}$ at pH 7, and 3.3 $\text{PO}_4 \text{ mg}\cdot\text{L}^{-1}\cdot 24 \text{ h}^{-1}$ at pH 8

Table 3. The results of the *t*-test on the quantity of inosinic acid (IMP) with the addition of various salts.

Salt added	Predicted value ($\mu\text{mol}\cdot\text{g}^{-1}$)				
	Day 0	Day 1	Day 2	Day 3	Day 4
None	9.5±0.86	8.0±0.69	7.2±1.3	7.3±0.37	4.6±2.2
NaCl	9.5±0.86	8.3±0.64	7.7±0.97	7.8±0.40*	5.7±1.8
MgCl ₂	9.5±0.86	8.7±0.64*	8.3±0.64	8.4±0.51**	6.9±1.4*
CaCl ₂	9.5±0.86	8.6±0.64	8.2±0.70	8.2±0.48**	6.7±1.5*
MgSO ₄	9.5±0.86	8.6±0.64	8.2±0.69	8.3±0.48**	6.7±1.4*
Na ₂ SO ₄	9.5±0.86	8.8±0.66*	8.5±0.58*	8.5±0.55**	7.3±1.3**

Mean values of nine independent determinations ($n = 9$). Most mean predictive values of IMP indicate significant differences ($p < 0.05$) after Day 3. * $p < 0.05$. ** $p < 0.01$ (*t*-test).

Discussion

In this study, the amount of IMP decreased significantly when fresh fish was stored at 4 °C over a 4 day period. Most of the ATP degraded within several hours (Japan Society of Refrigerating and Air Conditioning Engineers 2013). There was no increase in the IMP amount in this study, indicating that all ATP had degraded on day 0. Aubourg et al. (2007) and Rodríguez et al. (2009) reported that the quality of coho salmon raised in aquaculture facilities changed during storage. The decrease in the amount of IMP over time was also reported by Aubourg et al. (2007); in their study, IMP decreased from 5 to 3 $\mu\text{mol}\cdot\text{g}^{-1}$ over the course of 5 days. Although Rodríguez et al. (2009) used methodology that was similar to that of Aubourg et al. (2007) and to that used in the present study, maintaining fish samples at 2 °C, they reported little difference in the amount of IMP between days 0 and 5. One explanation might be that the levels of IMPase were low in their study.

After the death of fish, lactic acid increases in the flesh due to glycolysis of the glycogen in the muscles, and the pH of the fish muscles decreases (GMO Media, Inc. 2013). Koseki et al. (2006) reported that the decrease of pH in fish muscles generates H⁺, as well as lactate, as ATP degradation products. Because the amount of glycogen is low (<0.4%) in white benthic fish such as coho salmon, the pH value is 6.0-6.4. The glycogen level is approximately 1.0% in red migratory fish flesh, and the pH value is 5.2-5.6 (Koseki et al., 2006). The amount of IMPase activity markedly depends on pH value. The pH of the fish sample on the first day reported by Rodríguez et al. (2009) was 6.6±0.04, which was slightly higher than that (pH 6.0–6.2) observed in the present study or in the study of Aubourg et al. (2007).

In this study and that of Aubourg et al. (2007), the pH of fish flesh was 6.0-6.2, and the pH value was closely correlated with enzymatic activity, however, Rodríguez et al. (2009) reported a pH

of 6.6-6.7. Therefore, our findings are consistent with those of Aubourg et al. (2007) and unlike those of Rodríguez et al. (2009), suggesting that IMP was degraded earlier than that reported by Rodríguez et al. (2009).

In this study, the IMPase activity significantly increased after 24 h. Yokoyama et al. (1995, 1996) reported that IMP is degraded to HxR by bacteria. Since bacteria multiply exponentially, one explanation for the sudden increase in enzymatic activity could be that the bacterial population began multiplying 24 h after the reaction started. Since other factors besides enzyme activity might influence IMP degradation after the 24 h period, the degradation of IMP alone cannot be used to measure the enzyme activity. However, there was good enzymatic activity correlation with reaction time of up to 24 h after the start of the reaction and since the reaction velocity remained constant until this time, we selected the 24 h time point to measure IMPase activity.

The properties of IMPase differ across different fish species. Tomioka and Endo (1984) investigated the dependence of IMPase activity on pH in various fish species, including carp, *Cyprinus carpio* Linnaeus 1758, cod, *Gadus macrocephalus* (Tilesius 1810), common mackerel, *Scomber japonicus* Houttuyn 1782, Japanese horse mackerel, *Trachurus japonicus* (Temminck & Schlegel 1844), albacore, *Thunnus alalunga* (Bonnaterre 1788), silver whiting, *Sillago japonica* Temminck & Schlegel 1843, red seabream, *Pagrus major* (Temminck & Schlegel 1843), and sardine, *Sardinops melanostictus* (Temminck & Schlegel 1846). However, their results were inconsistent with those of the present study. Fish species that showed a similar dependence of IMPase on pH were carp, cod, and common mackerel all showing high levels of activity at pH 6-8), and Japanese horse mackerel, albacore, and silver whiting all showing high levels of activity at pH 5. Nedachi and Hirota (1991) reported that the IMPase activity of New Zealand golden snapper, *Pagrus auratus* (Forster 1801) also known as the silver sea bream, was very high at pH 6, which is similar to the findings in coho salmon, although the two species are not closely related.

As mentioned earlier, there are several forms of IMPase. Enzymes are easily affected by the external environment, particularly by pH and ionic strength. Therefore, their activity levels might change under different experimental conditions. For example, although red sea bream and New Zealand golden snapper are closely related genetically, the IMPase activity of the former was high at pH 4 (Tomioka and Endo 1984) and that of the latter was high at pH 6 (Nedachi and Hirota 1991). Apparently, the IMPase activity is also affected by the conditions of measurement and the extraction process used. In this study, the relative activity of IMPase at pH 6 was 40% in maleate buffer and 100% in succinic acid buffer, suggesting that the buffer influenced IMPase activity.

The tendency of IMPase activity to decrease at higher pH was shown by the addition of NaCl and CaCl₂ (NaCl: $p < 0.05$, CaCl₂: $p < 0.05$). The IMPase activity at pH 7-8 decreased by approximately 30% of its level at pH 4-5. Ooizumi et al. (2012) investigated the influence of NaCl on IMPase activity in horse mackerel and flathead flounder, *Hippoglossoides dubius* Schmidt 1904 at pH 6 and 7. Their findings were consistent with the present study where the IMPase activity was

lower at pH 7 than at pH 6 in horse mackerel. However, the IMPase activity was higher at pH 7 than at pH 6 in flathead flounder, confirming that IMPase properties differ across fish species. Oba et al. (1993) compared the IMPase activity of walleye pollock and silver whiting in a solution that contained no salts with that in a solution with various salts (NaCl, MgCl₂, or CaCl₂). At pH 7.5, the relative activity level was 7-12% with the addition of CaCl₂, 48% for NaCl, and 54-58% for MgCl₂. These results are consistent with those of our study.

IMPase activity barely changed and remained around 50% with the addition of MgCl₂ at various pH levels; therefore, MgCl₂ does not seem to have a strong influence on IMPase activity, although it could suppress IMPase activity at all pH levels.

IMPase activity increased with the addition of MgSO₄ with increasing pH, particularly at pH 7-8. The relative activity with the addition of MgSO₄ was low, and MgSO₄ suppressed IMPase activity at pH 4-5. IMPase activity decreased with the addition of Na₂SO₄, and the highest relative activity level was only 40-52% at pH 6. The relative activity decreased with the addition of MgSO₄ and Na₂SO₄ at pH 4-5 but increased with the addition of NaCl, MgCl₂, and CaCl₂ at pH 4-5. The results showed that SO₄²⁻ has a strong effect to suppress IMPase activity at pH 4-5. Therefore, the addition of MgSO₄ and Na₂SO₄ at pH 4-5; MgCl₂, CaCl₂, MgSO₄, or Na₂SO₄ at pH 6; and NaCl, CaCl₂, or Na₂SO₄ at pH 7-8 are expected to suppress IMPase activity in coho salmon.

The amount of IMP in the presence of MgCl₂ and Na₂SO₄ on day 1 and Na₂SO₄ on day 2 differed significantly from that observed when no salts were added ($p < 0.05$). The amount of IMP on days 3 and 4 also differed significantly between samples in the presence and absence of different salts, except in the case of NaCl addition at day 4 ($p < 0.05$, $p < 0.01$). Therefore, salts are effective at maintaining the amount of IMP in fish, and Na₂SO₄ was particularly effective for the storage of coho salmon.

Conclusion

In this study, we investigated the effect of various salts on the taste component of stored coho salmon flesh and found that salted coho salmon can be preserved longer than non-salted coho salmon. The amount of IMP variation from days 0 to 5 after the death of coho salmon was pH-dependent and affected by the addition of various salts. The IMPase activity of coho salmon was the highest at pH 6 and decreased sharply at pH 7-8. The effective preservation of IMP with the different salts and pH levels tested were in the following order: MgSO₄ or Na₂SO₄ at pH 4-5; MgCl₂, CaCl₂, MgSO₄, or Na₂SO₄ at pH 6; and NaCl, CaCl₂, or Na₂SO₄ at pH 7-8. As expected, IMP was preserved by the addition of various salts, and the amount of IMP remaining after 3 days was significantly higher when salts were added. In the future, we intend to evaluate the skin and colour of fish muscle after the addition of various salts along with quantity of IMP in fish preserved in salt for comparison with the predicted values.

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References

- Aubourg, S.P., V. Quiral, M.A. Larrain, A. Rodríguez, J. Gomez, L. Maier and J. Vinaigre. 2007. Autolytic degradation and microbiological activity in farmed coho salmon (*Oncorhynchus kisutch*) during chilled storage. Food Chemistry 104:369-375.
- GMO Media, Inc. (undated) Freshness maintenance manual of fishery products. <http://moon.ap.teacup.com/imorimasuda/html/1607sendohoji.pdf>. Accessed 1 November 2013.
- Japan Aquatic Oil Association. 2012. Statistical yearbook on aquatic oil. Japan Aquatic Oil Association, Tokyo. 61 pp.
- Japan Society of Refrigerating and Air Conditioning Engineers. (undated) ATP related compounds. <http://www.jsrae.or.jp/annai/yougo/152.html>. Accessed 18 November 2013.
- Koseki, S., S. Kitakami, N. Kato and K. Arai. 2006. Rigor mortis of fish and shellfish and evaluation of freshness of their muscles as K value. Journal of the School of Marine Science and Technology 4:31-46.
- Koseki, Y. 2013. The profile of salmon department fish-11. Fisheries Research Agency Salmonid Research Report No. 7:34-37.
- Nagasawa, T. 2013. Fishery and resources investigation of salmon and trout (review paper). The Present Situation of the International Fishery Resource No. 56:1-6.
- Nara, K.. 1992. Current salmon aquaculture in the world. Fish and eggs. Technical reports of Hokkaido Salmon Hatchery 161:59-68. http://salmon.fra.affrc.go.jp/kankobutu/tech_repo/fe01/fishandegg161_p059-068.pdf. Accessed 19 February 2014.
- Nedachi, K. and N. Hirota. 1991. Changes in ATP related compounds and IMP-degrading enzyme activity of New Zealand golden snapper (*Pagrus auratus*). Nippon Suisan Gakkaishi 57:329-335.
- Niino, Y., H. Nishimura, A. Koga, Y., Nakayama and M. Haga. 2003. Quality of common salt (part II). Journal of Cookery Science of Japan 36:305-320.
- Oba, K. and E. Niwa. 1993. The mode of inhibition by salts to two enzymes involved in IMP degradation in fish flesh. Journal of Food Science and Technology 40:583-588.
- Ooizumi, T., Y. Hibino and M. Matsukawa. 2012. Fish species specificity on the salt-independent of the IMPase activity in fish muscle. Collaborative Investigation about the Use of Marine Products 52:3-7.
- Oka Foods Co., Ltd. 2012. "The manufacturing method and cooking method of the frozen fish," Japanese Patent Disclosure P2012-14776A.

Perrin, D.D. and B. Dempsey. 1981. Buffers for pH and metal ion control. pp 138-140. Chapman and Hall, London.

Rodríguez, A., N. Carriles, J.M. Gallardo and S.P. Aubourg. 2009. Chemical changes during farmed coho salmon (*Oncorhynchus kisutch*) canning: Effect of a preliminary chilled storage. *Food Chemistry* 112:362-368.

Salt Industry Center of Japan. 2007. Analysis method of the salt. pp. 60-61 <http://www.shiojigyo.com/english/>. Accessed 25 November 2013.

Srirangsan, P., N. Hamada-Sato, K. Kawai, M. Watanabe and T. Suzuki. 2010. Improvement of fish freshness determination method by the application of amorphous freeze-dried enzymes. *Journal of Agricultural and Food Chemistry* 58:12456-12461.

Tomioka, K. and K. Endo. 1984. K-value increasing rates and IMP-degrading activities in various fish muscles. *Nippon Suisan Gakkaishi* 50:889-892.

Tomioka, K. and K. Endo. 1986. Degradation of inosinic acid in fish flesh during the preparatory treatments for cooking. *Journal of Cookery Science of Japan* 19:289-294.

Yokoyama, Y., M. Sakaguchi, Y. Azuma, F. Kawai and M. Kanamori. 1995. ATP-breakdown by endogenous enzymes in the mantle of oyster during storage. *Annual Report of the Environmental Science Research Institute* 14:73-77.

Yokoyama, Y., M. Sakaguchi, Y. Azuma, F. Kawai and M. Kanamori. 1996. Postmortem changes of ATP and its related compounds in oyster tissues in the presence of antibiotic chloramphenicol. *Fisheries Science* 62:312-316.

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