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Autolytic degradation and microbiological activity in farmed Coho salmon (*Oncorhynchus kisutch*) during chilled storage

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Abstract

Two deteriorative pathways (autolysis and microbiological activity) were studied in farmed Coho salmon (*Oncorhynchus kisutch*) for 24 days during chilled storage. These changes were assessed by nucleotide degradation (determination of adenosine 5′-triphosphate and its degradation compounds) and biochemical (pH; content of total volatile base-nitrogen; trimethylamine-nitrogen, TMA-N; histamine) and microbial (total aerobe mesophiles, TAM; coliforms) indices related to bacterial activity. An important nucleotide degradation could be assessed according to the fast inosine 5′-monophosphate formation, followed by degradation into inosine and hypoxanthine; the *K* value was found to be an accurate tool for the measurement of quality loss throughout the whole experiment. Regarding bacterial activity, contents of TMA-N and histamine and TAM counts assessment showed sharp increases after the end of the microbial lag phase (12–17 days); however, values obtained for histamine content and TAM growth remained below acceptable security limits throughout the whole experiment.
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1. Introduction

Marine foods have attracted a great deal of attention from consumers as a source of high amounts of important nutritional components that could lead to a positive role on human health and nutrition (Illingworth & Ullmann, 1990; Simopoulos, 1997). However, in recent years, the fishing sector has suffered from dwindling stocks of traditional species as a result of dramatic changes in their availability. This has prompted fish technologists and the fish trade to pay more attention to aquaculture techniques as a source of fish and other seafood products (FAO, 2000; Hew & Fletcher, 2001).

Wild and farmed chilled fish species deteriorate rapidly *post-mortem* due to the effects of autolytic degradation, microbial spoilage and lipid oxidation, that lead to a detrimental effect on the commercial value of fish products (Ashie, Smith, & Simpson, 1996; Olafsdóttir et al., 1997; Whittle, Hardy, & Hobbs, 1990). The rate of alteration has been shown to depend on factors such as the nature of the fish species, size, lipid content, feeding state at the moment of capture, importance and nature of microbial load and storage temperature.

Among cultivated fish, Coho salmon (*Oncorhynchus kisutch*), also called silver salmon, has acquired great attention because of its increasing production in countries such as Chile, Japan and Canada (FAO, 2005a), in parallel with important capture production in countries such as USA, Russian Federation, Canada and Japan (FAO,

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2005b). Related to this fish species, most research has been carried out on genetic aspects and farming conditions during the aquaculture production (Estay, Díaz, Neira, & García, 1997; Winkler, Bartley, & Díaz, 1999). However, compositional or technological research appears to be scarce, accounting for studies of fatty acid distribution (Gruger, Nelson, & Stansby, 1964; Romero, Robert, Masson, & Pineda, 1996) and lipid changes related to quality loss during chilled (Aubourg et al., 2005b) and frozen (Braddock & Dugan, 1969) storage. The present work focusses on the autolytic degradation and microbial activity of Coho salmon and its commercialisation as a chilled product. Different biochemical and microbiological indices were studied throughout a 24-day storage period.

2. Materials and methods

2.1. Raw material, processing, sampling and chemicals

Specimens of farmed Coho salmon (*O. kisutch*) were obtained from EWOS Innovation Research (Colaco, Puerto Montt, Chile). Fish specimens (weight range: 3.0–3.4 kg) were sacrificed by a sharp blow to the head, the gills cut, bled in a water–ice mixture, headed, gutted and kept in ice for 16–20 h until they arrived at our laboratory. At that moment, the fish specimens were stored on ice in an isothermal room at 2 °C. Samples were taken for analysis on days 0, 3, 6, 10, 12, 17, 19 and 24. Five different individuals ($n = 5$) were considered and analysed (by day studied) separately to achieve the statistical analysis.

Chemicals employed during the present work (solvents, reagents) were reagent grade (E. Merck; Darmstadt, Germany).

2.2. Biochemical analyses

Nucleotide extracts were prepared according to the method of Ryder (1985) and stored at −30 °C prior to analysis. Nucleotide analysis was performed by HPLC as previously described (Aubourg, Piñeiro, Gallardo, & Barros-Velázquez, 2005a). Standard curves for adenosine 5′-triphosphate (ATP) and each compound involved in its degradation pathway, adenosine 5′-diphosphate (ADP), adenosine 5′-monophosphate (AMP), inosine 5′-monophosphate (IMP), inosine (INO) and hypoxanthine (HX), were constructed in the 0–1 mM range. Results obtained for each degradation compound were expressed as mmol kg^{-1} muscle. The K value was calculated according to the following concentration ratio: K value (%) = 100 × (HX + INO)/(ATP+ADP+AMP+IMP+INO+HX).

The evolution of pH values in Coho salmon muscle during the storage time was determined by means of a 6 mm diameter insertion electrode (Crison, Barcelona, Spain).

Total volatile base-nitrogen (TVB-N) contents were measured according to some modifications of the Antonacopoulos (1960) method. Briefly, fish muscle (10 g) was extracted with 6% perchloric acid and brought up to 50 ml. TVB-N contents were determined, after steam-distillation of the acid extracts rendered alkaline to pH 13 with 20% NaOH, by titration of the distillate with 10 mM HCl. The results are expressed as mg TVB-N kg^{-1} muscle.

Trimethylamine-nitrogen (TMA-N) values were determined by the picrate method, as previously described (Tozawa, Erokibara, & Amano, 1971). This involves the preparation of a 5% trichloroacetic acid extract of fish muscle (10 g/25 ml). Results are expressed as mg TMA-N kg^{-1} muscle.

Histamine content in fish muscle was determined by capillary zone electrophoresis, according to the Gallardo, Sotelo, and Pérez-Martín (1997) method. This involves the preparation of a 6% perchloric acid extract of fish muscle (5 g/20 ml). Results are expressed as mg histamine kg^{-1} muscle.

2.3. Microbiological analysis

Samples of 5 g of fish muscle were aseptically skinned and dissected from chilled salmon specimens by means of sterile surgical blades under sterile air-flow conditions to avoid cross-contamination. Samples were then mixed with 45 ml of peptone water (1 g l^{-1}) and homogenised in a stomacher (Seward Medical, London, UK). Serial dilutions from the microbiological extracts were prepared in peptone water. Total aerobic mesophiles (TAM) were investigated in plate count agar (PCA, Oxoid Ltd., London, UK) after incubation at 31 °C for 72 h (Ben-Gigirey, Vieites, Villa, & Barros-Velázquez, 1998). Coliforms were investigated in Violet Red Bile Agar (VRBA medium, Merck, Darmstadt, Germany) after incubation at 30 ± 1 °C for 24 ± 2 h, as recommended by the manufacturer (Merck Microbiology Manual, 2002). Samples of chilled salmon were processed for *Salmonella* sp., *Listeria* sp. and *Staphylococcus aureus*, following standard laboratory procedures as described by Jay (1994).

2.4. Statistical analyses

Data from the different biochemical and microbiological analyses were subjected to one-way analysis of variance ($p < 0.05$). Correlation analyses among chilled time, biochemical indices and microbiological parameters were studied (Statsoft, 1994).

3. Results and discussion

3.1. Nucleotide degradation

According to general knowledge, the present study revealed a rapid degradation to IMP after death, followed by a progressive degradation to INO and HX throughout the experiment time. Accordingly, evolution of IMP, INO and HX contents was studied, their results being expressed in Fig. 1.
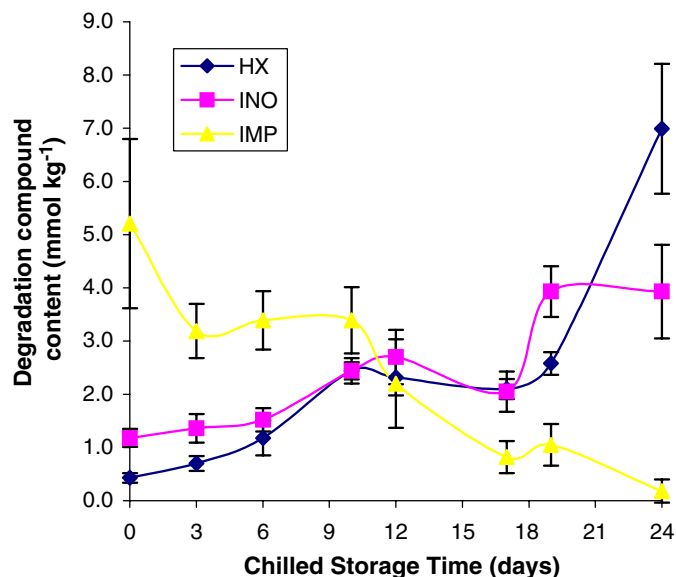
Fig. 1. Evolution of inosine 5′-monophosphate (IMP), inosine (INO) and hypoxanthine (HX) contents (mmol kg⁻¹ fish muscle) (Bars denote standard deviation of the mean ($n = 5$)) in Coho salmon muscle during chilled storage.

A decrease ($p < 0.05$) with time was observed for the IMP content ($r^2 = -0.87$; Table 1), its value being negligible at the end of the experiment. This decrease was markedly important at day 3 and then at day 17. IMP is recognised as a flavour enhancer of meaty foods, especially the umami flavour (Kawai, Okiyama, & Ueda, 2002), and it is likely that IMP contributes to the sweet, creamy, meaty flavours of fresh fish. Present results concerning the progressive IMP loss agree with previous research (Kyrana & Lougovois, 2002; Ryder, Buisson, Scott, & Fletcher, 1984) and show a freshness loss with chilled time.

INO and HX contents (Fig. 1) provided non-regular pattern increases during storage, so that very good correlation values with time were not obtained ($r^2 = 0.84$ and $r^2 = 0.88$, respectively; Table 1). This non-regular formation could be explained because of the different pathways involved, since degradation of IMP to INO and HX is

reported to be catalysed mainly by endogenous IMP phosphohydrolase and INO ribohydrolase, with a contribution from bacterial enzymes as storage time increases (Howgate, 2005). In the present experiment, INO content showed sharp increases ($p < 0.05$) at days 10 and 19, and HX showed sharp increases ($p < 0.05$) at days 10 and 24. A significant difference between molecule contents could not be found ($p > 0.05$) throughout the experiment, which agrees with previous research on Atlantic salmon (Erikson, Beyer, & Sigholt, 1997). Bibliography accounts for species (albacore, New Zealand jack mackerel and European sea bass) that show higher INO content than HX content throughout the whole experiment (Kyrana & Lougovois, 2002; Pérez-Villarreal & Pozo, 1990; Ryder et al., 1984); on the contrary, other species, such as rockfish (Mendes, Quinta, & Nunes, 2001) and rainbow trout (Howgate, 2005), at the end of the chilled storage showed a higher content of HX than of INO.

Due to the wide range of differences among fish species concerning the ATP degradation mechanism, the K value has been proposed as a good index for assessing ATP degradation and, accordingly, the freshness loss. In the present experiment, the K value was studied (Fig. 2) and provided a progressive and regular increase during the storage time ($r^2 = 0.97$, linear fitting; Table 1), reaching values of about 95–98% at the end of the experiment. A good inverse ratio between the K value and the IMP content was observed ($r^2 = -0.92$; Table 2) but not with INO and HX contents ($r^2 = 0.79$ and $r^2 = 0.78$, respectively; Table 2), which agrees with the above-mentioned non-regular pattern increases found for both metabolites.

A great difference has been reported for the K value development in different fish species throughout chilled storage. Thus, a fast increase with time for the K value has been obtained for cod (Ehira & Uchiyama, 1974) and rockfish (Mendes et al., 2001), while values above 80% were not obtained at the end of the chilled time for species such as turbot (Aubourg, Piñeiro, Gallardo, et al., 2005), New Zealand jack mackerel (Ryder et al., 1984) or European
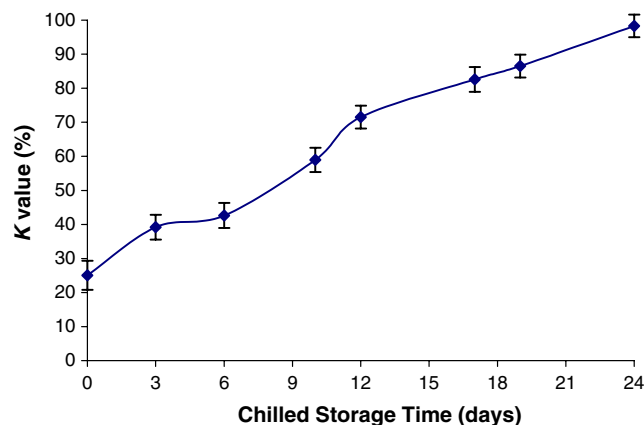
Table 1
Correlation values (CV) between the chilled storage time and the different parameters analysed[A]

Parameter	CV
Inosine 5′-monophosphate	**−0.87**[a]
Inosine	**0.84**[a]
Hypoxanthine	**0.88**[b]
K value (%)	**0.97**[a]
pH	− 0.27[c]
Total volatile base-nitrogen	**0.56**[a]
Trimethylamine-nitrogen	**0.97**[b]
Histamine	**0.93**[b]
Aerobe mesophile counts	**0.78**[b]

[A] For each index, linear[a], quadratic[b] and logarithmic[c] fittings were studied. In each case, the best correlation value is expressed. Significant values ($p < 0.05$) are given in bold.

Fig. 2. K value (%) assessment (Bars denote standard deviation of the mean ($n = 5$)) in Coho salmon muscle during chilled storage.

Table 2
Linear correlation values among the different parameters[a] studied concerning autolytic degradation and microbiological activity[b]

	INO	HX	K value	pH	TVB-N	TMA-N	HIS	TAM
IMP	**−0.77**	**−0.68**	**−0.92**	0.16	**−0.56**	**−0.76**	**−0.74**	**−0.64**
INO		**0.77**	**0.79**	**−0.16**	**0.46**	**0.81**	**0.72**	**0.79**
HX			**0.78**	**−0.19**	0.31	**0.89**	**0.77**	**0.68**
K value				−0.18	**0.60**	**0.84**	**0.79**	**0.70**
pH					−0.08	−0.22	−0.28	−0.17
TVB-N						**0.47**	**0.45**	**0.40**
TMA-N							**0.89**	**0.79**
HIS								**0.79**

[a] Abbreviations: IMP (inosine 5′-monophosphate), INO (inosine), HX (hypoxanthine), TVB-N (total volatile base-nitrogen), TMA-N (trimethylamine-nitrogen), HIS (histamine) and TAM (total aerobe mesophile counts).
[b] Significant values ($p < 0.05$) are given in bold.

sea bass (Kyrana & Lougovois, 2002). In salmonid species, a sharp increase has been reported during the first days of storage, followed by a levelling-off period so that, again, values above 80% are not obtained; such conclusions apply to Atlantic salmon (*Salmo salar*; Erikson et al., 1997), rainbow trout (*Oncorhynchus mykiss*; Boyle, Lindsay, & Stuiber, 1991), sockeye salmon (*Oncorhynchus nerka*; Luong, Male, & Huynh, 1991) and trout (*Salmo trutta*; Hattula & Kiesvaara, 1992).

3.2. Microbiological activity: biochemical indices

The pH value did not cause differences throughout the storage period (Table 3), showing relatively low values that were included in the 6.00–6.25 range. The pH value has generally shown an important increase in later stages of chilled experiments according to the end of the microbial lag phase. Such behaviour could be observed in fish species under similar storage conditions, such as sardine (Nunes, Batista, & Morâo de Campos, 1992), European sea bass (Kyrana & Lougovois, 2002), New Zealand mackerel (Ryder et al., 1984), turbot (Rodríguez, Barros-Velázquez, Ojea, Piñeiro, & Aubourg, 2003) and horse mackerel (Rodríguez, Losada, Aubourg, & Barros-Velázquez, 2005).

When volatile amine formation was studied, as a whole, few differences could be found during the present storage (TVB-N; Table 3). Compared to the starting fish value, a significant increase could only be obtained at day 17, with no further increase until the end of the experiment; thus, a

poor correlation value with time was observed ($r^2 = 0.56$; Table 1). According to the above-mentioned pH value, starting TVB-N content and TVB-N formation during the present experiment can be considered very low compared to other fish species kept under the same conditions, such as sardine (Nunes et al., 1992), European sea bass (Kyrana & Lougovois, 2002) and albacore (Pérez-Villarreal & Pozo, 1990).

However, trimethylamine (TMA-N; Table 3) formation proved to be a more accurate quality index than did TVB-N, since a good correlation value with time was obtained ($r^2 = 0.97$; Table 1). A quadratic fitting was obtained, showing the lag phase end-effects at day 12; after that time, further TMA-N increases could be obtained until the end of the experiment. Starting fish values, as well as values obtained at the end of the experiment, can be considered high when compared to species such as albacore (Pérez-Villarreal & Pozo, 1990) or European sea bass (Kyrana & Lougovois, 2002); however, the present results were in the same range as those for sardine (Nunes et al., 1992), hake (Ruiz-Capillas & Moral, 2001a) and horse mackerel (Rodríguez et al., 2005) and even lower than those observed for herring and haddock (Fernández-Salguero & Mackie, 1987). A poor correlation value ($r^2 = 0.47$; Table 2) was obtained between the two amine indices (TVB-N and TMA-N).

Most previous research has shown that TVB-N and TMA-N remain below discrete values until the lag phase is finished. Thus, small-size fish species, such as sardine

Table 3
Assessment of different quality indices[A] in Coho salmon muscle during chilled storage[B]

Chilled time (days)	pH	TVB-N	TMA-N	Histamine
0	6.17 a (0.05)	112 a (19.4)	23.7 ab (6.7)	0.0 a (0.0)
3	6.17 a (0.04)	130 ab (16.3)	22.5 a (1.5)	0.0 a (0.0)
6	6.09 a (0.04)	148 ab (16.6)	26.8 ab (1.9)	0.0 a (0.0)
10	6.10 a (0.04)	151 ab (16.0)	28.4 b (3.4)	2.3 a (2.3)
12	6.21 a (0.04)	165 ab (15.1)	36.8 c (5.9)	0.0 a (0.0)
17	6.13 a (0.04)	175 b (16.4)	44.1 d (3.9)	28.8 b (11.7)
19	6.13 a (0.04)	191 b (15.0)	56.8 e (6.4)	36.1 b (8.4)
24	6.09 a (0.04)	169 ab (15.0)	80.4 f (4.1)	46.4 c (13.6)

[A] Abbreviations: TVB-N (total volatile base-nitrogen) and TMA-N (trimethylamine-nitrogen).
[B] In each column, mean values ($n = 5$) followed by different letters are significantly ($p < 0.05$) different. Standard deviations are indicated in brackets.

(Nunes et al., 1992), mackerel (Bennour, El Marrakchi, Bouchriti, Hamama, & El Ouadaa, 1991) and horse mackerel (Rodríguez et al., 2005) have shown a 7–9 day period, while higher-size fish species, such as albacore (Pérez-Villarreal & Pozo, 1990), redfish (Rehbein, Martinsdóttir, Blomsterberg, Valdimarsson, & Oehlenschläger, 1994) and turbot (Rodríguez et al., 2003) have shown higher lag phase times that agree with the TVB-N and TMA-N values obtained in the present experiment.

Histamine formation was negligible in the 0–12 day period (Table 3). After that time, and according to the lag phase end, a sharp content increase was produced until the end of the experiment, according to previous research on chilled sardine (Gallardo et al., 1997), mackerel (Bennour et al., 1991) and herring and haddock (Fernández-Salguero & Mackie, 1987). However, even at day 24, values obtained are below the acceptability limit (100 mg kg^{-1} muscle) (Anon, 1991; SERNAPESCA, 2005). As in the case of trimethylamine formation, a good quadratic fitting with time could be obtained ($r^2 = 0.93$; Table 1), showing histamine to be an accurate tool for assessing quality loss. Both amine indices (TMA-N and histamine) showed a fair correlation value ($r^2 = 0.89$; Table 2), according to previous research (Ruiz-Capillas & Moral, 2001b).

3.3. Microbiological activity: microbiological indices

Total aerobic mesophile bacteria were analysed during the storage (Fig. 3). Their content did not show changes in the 0–10 day period. From this day on, a significant increase of bacterial counts was verified ($p < 0.05$), followed by no changes in the 19–24 day period. However, even the results obtained in the last storage period (days 19–24) can be considered to be within the acceptable limits for export fishing products (Gram & Huss, 1996; SERNAPESCA, 2005). When compared to other parameters, aerobic mesophile growth showed the best correlation value with TMA-N and histamine contents ($r^2 = 0.79$ in
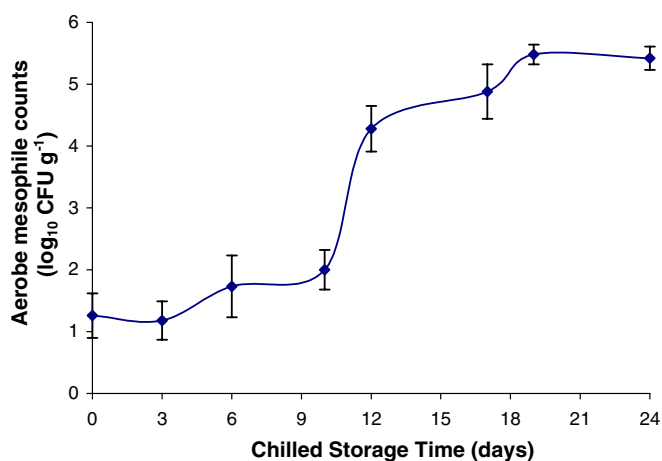
both cases; Table 2); a similar correlation value ($r^2 = 0.78$; Table 1) was obtained with chilled time.

A higher aerobic mesophile development was obtained in small-size species, such as Atlantic mackerel (Bennour et al., 1991; Jhaveri, Leu, & Constantinides, 1982), European sea bass (Kyrana & Lougovois, 2002), horse mackerel (Rodríguez et al., 2005) and New Zealand mackerel (Ryder et al., 1984) than in the present study. However, medium-size species, such as turbot (Rodríguez et al., 2003) and Atlantic salmon (Sveinsdóttir, Martinsdóttir, Hyldig, Jørgensen, & Kristbergsson, 2002) showed behaviour similar to Coho salmon.

Microbiological analyses of the chilled fish confirmed the absence of Salmonella sp. and Listeria sp. throughout the whole experiment. The fecal coliform and S. aureus counts did not show differences ($p > 0.05$) during the study, being less than 3 MPN g^{-1} and less than 10 CFU g^{-1}, respectively, in all cases.

4. Conclusions

The evolution of two deteriorative pathways (autolytic degradation and microbiological spoilage) was studied during chilled storage of Coho salmon. Autolytic degradation proved to be fast, leading to a negligible content of IMP at the end of the experiment, so that the K value reached 95–98%.

Microbiological activity proved to be low. Thus, indices such as pH and TVB-N were low throughout the experiment while others, e.g. TMA-N, histamine and total aerobic mesophile bacteria, showed important increases with time, although values remained in all cases within the acceptable security limits until the end of the experiment.

In a previous work (Aubourg, Vinagre, Rodríguez, et al., 2005), rancidity development was assessed during Coho salmon chilled storage by biochemical and sensory analyses. According to the present results on microbiological activity, the lipid damage developed slowly, so that sensory analyses showed non-acceptable rancid odour at day 19. Both damage pathways (microbiological activity and rancidity development) are responsible for the shelf-life time of chilled fatty fish species (Ashie et al., 1996; Whittle et al., 1990). The slow damage development found in Coho salmon agrees with results obtained for medium-size fatty fish species, such as albacore (Pérez-Villarreal & Pozo, 1990) and Atlantic salmon (Sveinsdóttir et al., 2002), while small-size fatty fish species, such as sardine (Nunes et al., 1992), mackerel (Bennour et al., 1991; Jhaveri et al., 1982) and horse mackerel (Rodríguez et al., 2005), show rapid deterioration.

Fig. 3. Evolution of total aerobe mesophile growth (log$_{10}$ CFU g^{-1} fish muscle) (Bars denote standard deviation of the mean ($n = 5$)) in Coho salmon muscle during chilled storage.

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