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Chemical changes during farmed coho salmon (*Oncorhynchus kisutch*) canning: Effect of a preliminary chilled storage

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ABSTRACT

A relevant farmed fish species (coho salmon; *Oncorhynchus kisutch*) was studied as a raw material for the canning process. The effects of preliminary chilling storage and thermal treatment (cooking and sterilisation) on the chemical constituents (lipids and non-protein nitrogen compounds) of the canned fish were analysed. An increasing previous chilling time led to an important autolysis (*K* value) development, and to an increasing formation of free fatty acids, and interaction compounds (fluorescence and browning assessments) ($p < 0.05$) in the canned product. The thermal treatment led to the formation of volatile amines (total and trimethylamine), free fatty acids, secondary lipid oxidation compounds (anisidine and thiobarbituric acid values) and interaction compounds in canned fish. Interaction compound assessment was found the most useful tool to study the lipid oxidation and non-enzymatic browning developments, while the *K* value showed to be an interesting index for assessing the freshness stage of the raw material employed.

1. Introduction

Marine species provide high content of important constituents for the human diet such as nutritional and digestible proteins, lipid-soluble vitamins (A and D, namely), microelements (I, F, Ca, Cu, Zn, Fe, Se and others) and ω-3 polyunsaturated fatty acids (Simopoulos, 1997). However, such species led to highly perishable food products whose freshness and quality rapidly decline post-mortem. Thus, different damage pathways are known to develop after death that can be summarised as endogenous enzyme activity, microbiological development, non-enzymatic lipid oxidation and browning, and enzymatic browning (Pigott & Tucker, 1990). The relative incidence of each damage mechanism will depend on the kind of technological process applied and on the kind of the marine species involved.

Canning is one of the most important means of fish preservation (Horner, 1997). In it, two thermal steps (cooking and sterilisation) are included, so that both enzymes and bacteria should be permanently inactivated by heat and, provided reinfection does not occur and no negative interaction with the container is produced, heat processed fish keeps for a very long time. However, canneries have to store the raw material as frozen or chilled before it is canned, so that many of the problems with canned fish can be related to the quality of the raw material employed, which continuously changes during storage (Aubourg & Medina, 1997).

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 (Stickney, 1990). Related to canning processing, aquaculture production can provide high quality starting raw fish as a result of avoiding most of the inconveniences arising from chilling and frozen storage conditions that are necessary to be applied by canneries for wild fish species.

The present study was focused on the employment of farmed coho salmon (*Oncorhynchus kisutch*) as raw material for canned product preparation. This species was chosen according to its increasing production in countries such as Chile, Japan and Canada (FAO, 2007a) in parallel to important capture production in countries like USA, Russian Federation, Canada and Japan (FAO, 2007b). In the actual work, the effects of a preliminary chilled storage and the thermal treatment (cooking and sterilisation) on the quality-related chemical constituents (lipids and non-protein nitrogen compounds) of the canned fish were analysed.

2. Materials and methods

2.1. Slurry ice preparation

In the present work, an advanced chilling system (slurry ice) was employed as a holding process (Losada, Piñeiro, Barros-Velázquez, &

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Aubourg, 2005). Therefore, a slurry ice prototype (FLO–ICE, Kinarca S.A.U., Vigo, Spain) was used. The composition of the slurry ice binary mixture was 40% ice/60% water, prepared from filtered seawater (salinity: 3.3%). The temperature of the slurry ice mixture was −1.5 °C. The average temperature of the specimens processed in slurry ice was in the range of −1.0 °C to −1.5 °C. Throughout the experiment, the slurry ice mixture was renewed when required.

2.2. Raw fish, sampling, chilling storage, and chemicals

Specimens (16 individuals) of farmed coho salmon (*O. kisutch)* (weight range: 2.8–3.2 kg) were obtained from an aquaculture farm (Comercial Xanquéi; Lousame, La Coruña, Spain) and were sacrificed at the aquaculture farm by immersion in slurry ice. Individuals were kept under this chilling condition during transportation to the laboratory. Upon arrival in the laboratory (day 0), the fish specimens were neither headed nor gutted, but directly placed in an isothermal room at 2 °C and were surrounded by slurry ice at a 1:1 fish to ice ratio.

On the starting day (day 0), four individuals were not thermally treated and were analysed to obtain control data. Also on day 0, four other individuals were thermally treated according to conditions expressed below. The remaining fish (eight individuals) were taken for the canning process on days 5 and 9 of chilled storage (four individuals at each time).

Previous research concerning the chilled storage of the present species (Aubourg et al., 2007) has shown that after a 10 days-period storage microbiological development (trimethylamine value and aerobe mesophile counts) increased largely. Accordingly, in the present experiment, three different chilling times below this time limit were chosen.

Both in raw and in canned samples, each individual fish was studied separately from others to achieve the statistical study ($n = 4$). Chemicals (solvents, reagents) used in the experiments were reagent grade (E. Merck; Darmstadt, Germany).

2.3. Thermal treatment

Specimens of the starting day (day 0) and those kept under chilling conditions (5 and 9 days) were steam cooked in a horizontal retort during 45 min in our pilot plant (102–103 °C) to a final backbone temperature of 65 °C; the fish were then cooled at room temperature (15–18 °C) for about 2 h, headed and eviscerated.

Muscle portions (90 g) from salmon specimens were placed in small flat rectangular cans (105 × 60 × 25 mm; 150 ml). Two grams of NaCl were weighed and added to each can that was then filled with sunflower oil as coating medium. The cans were vacuum-sealed and sterilised in a horizontal steam heated retort (115 °C, 45 min; $F_o = 7$ min). When the heating time was completed, steam was cut off and air was used to flush away the remaining steam. Cans cooling was carried out at reduced pressure.

After 3 months of storage at room temperature (15–18 °C), the cans were opened and the liquid part was carefully drained off gravimetrically, filtered by means of a filter paper and collected. Then, the resulting liquid phase was centrifuged, the oil phase separated and dried with anhydrous Na_2SO_4. Salmon muscle was wrapped in filter paper. Fish white muscle and the coating oil medium were used for chemical analyses. Initial oil and oil that was canned in the absence of salmon muscle were also analysed.

Canning manufacturers recommend not to open cans before a 2–3 months-period has elapsed. This time is considered necessary for an adequate homogenisation of components inside the can. According to this, a 3 months-storage period was employed in the present study.

2.4. Composition analyses

Moisture content was determined by weight difference between the homogenised fish muscle (1–2 g) before and after 24 h at 105 °C. Results were expressed as g water kg^{-1} muscle.

The lipid fraction was extracted from the fish muscle by the Bligh and Dyer (1959) method. Quantification results were expressed as g lipid kg^{-1} muscle.

NaCl contents were determined after boiling portions of fish muscle in the presence of HNO_3, followed by the addition of excess 0.1 N $AgNO_3$ and the titration of non-neutralised silver nitrate with 0.1 N NH_4SCN (AOAC, 1990). The results were expressed as g NaCl kg^{-1} muscle.

2.5. Nucleotide degradation analysis

Nucleotide extracts were prepared and analysed according to previous research (Aubourg, Piñeiro, Gallardo, & Barros-Velázquez, 2005). Nucleotide analysis was performed by HPLC, using a Beckman device provided with the programmable solvent module 126, and the scanning detector module 167 connected to the System Gold software, version 8.1 (Beckman). Separations were achieved on a reverse-phase Spherisorb ODS-2 C18 250 × 4.60 mm column (Waters), with an internal particle diameter of 5 μm. The composition of the mobile phase was as follows: solvent A was composed of 0.04 M KH_2PO_4 + 0.006 M K_2HPO_4, pH 7; solvent B was acetonitrile. Solvents were filtered through a 0.45 μm aqueous filter before use. Separations were carried out using a continuous gradient elution with both solvents. The eluent was monitored at 254 nm and the running time was 10 min.

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 calculated as mmol kg^{-1} muscle. The K value was calculated according to the following concentration ratio: K value (%) = 100 × (INO + HX)/(ATP + ADP + AMP + IMP + INO + HX).

2.6. Volatile amine formation and pH value evolution

Total volatile base-nitrogen (TVB-N) values were measured by a steam distillation method. For it, fish muscle (10 g) was extracted with 6% (w/v) perchloric acid and brought up to 50 ml, determining the TVB-N content – after steam-distillation of the acid extracts rendered alkaline to pH 13 with 2% (w/v) NaOH – by titration of the distillate with 10 mM HCl. The results were expressed as mg TVB-N kg^{-1} muscle.

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

The evolution of pH values in coho salmon muscle was determined by means of a 6-mm diameter insertion electrode (Crison, Barcelona, Spain).

2.7. Analysis of lipid degradation

Free fatty acid (FFA) content was determined by the Lowry and Tinsley (1976) method based on complex formation with cupric acetate–pyridine followed by spectrophotometric (715 nm) assessment. Results were expressed as g FFA kg^{-1} lipids.

Primary lipid oxidation was determined by means of the peroxide value (PV) according to the ferric thiocyanate method (Chap-

man & McKay, 1949). The results were expressed as meq active oxygen kg^{-1} lipids.

The anisidine value was determined in fish muscle according to the AOCS (1993) method, based on the reaction between α- and β-unsaturated aldehydes (primarily 2-alkenals) and *p*-anisidine reagent. Anisidine value was expressed as 100 times the absorbance measured at 350 nm in a 1 cm path length cuvette from a solution containing 10 mg lipid ml^{-1} reaction medium.

The thiobarbituric acid index (TBA-i) was determined according to Vyncke (1970). This method is based on the reaction between a trichloracetic extract of the fish muscle, and thiobarbituric acid at high temperature (95–97 °C), the resulting chromophore was measured at 532 nm. Results were expressed as mg malondialdehyde kg^{-1} fish muscle.

Lipid extracts from the fish muscle were converted into fatty acid methyl esters (FAME) by employing acetyl chloride and analysed by GC (Perkin-Elmer 8700 chromatograph) employing a fused silica capillary column SP-2330 (0.25 mm i.d. ×30 m, Supelco Inc., Bellefonte, PA, USA) (Aubourg & Medina, 1997). Carrier gas used was N_2 flowing with a linear velocity of 18 cm s^{-1}. A flame ionisation detector was set at 250 °C. Peaks were identified by comparison of their retention times with standard FAME mixtures (Larodan, Qualmix Fish; Supelco, FAME Mix). Peaks were automatically integrated, C19:0 fatty acid being used as internal standard for quantitative analysis. The polyene index (PI) was calculated as the following fatty acid ratio: PI = C20:5 + C22:6/C16:0.

2.8. Interaction compound formation

Formation of fluorescent compounds was determined with a Perkin Elmer LS 3B fluorimeter by measurements at 393/463 nm and 327/415 nm as previously described (Aubourg & Medina, 1997). The relative fluorescence (RF) was calculated as follows: $RF = F/F_{st}$, where F is the fluorescence measured at each excitation/emission maximum, and F_{st} is the fluorescence intensity of a quinine sulphate solution (1 μg ml^{-1} in 0.05 M H_2SO_4) at the corresponding wavelength. The fluorescence ratio (FR) was calculated as the ratio between the two RF values: $FR = RF_{393/463\ nm}/RF_{327/415\ nm}$. The FR value was determined in the aqueous phase resulting from the lipid extraction of the fish muscle and in the coating oil.

Non-enzymatic browning (NEB) development was determined spectrophotometrically at 420 nm in the lipid extract of the edible flesh and in the coating oil (Aubourg, Pérez-Martín, Medina, & Gallardo, 1992). The results were calculated using the equation: $NEB = A \times V/w$, where A is the absorbance reading at 420 nm, V is the volume (ml) of the sample and w is the amount (mg) of the lipid sample.

2.9. Sensory analysis

The development of rancid and putrid odours was conducted by a sensory panel consisting of ten experienced judges (5 males and 5 females; 25–55 years old). Panellists had been involved in sensory analysis of different kinds of fish foods during the last 10 years. Previously to the present experiment, a special training was carried out concerning canned salmon (fish muscle and coating oil) of different quality conditions.

At each sampling time, the fish muscle portions and coating oils were presented to the panellists in individual trays and were scored individually. The panel members shared samples tested. Rancid and putrid odour developments were evaluated on a hedonic scale with numerical scores from 0 (stage of no rancidity/putridity at all) to 10 (stage where no increase in rancidity/putridity is possible); score 5.0 was considered the borderline of fish acceptability. Scores among panellists were averaged.

2.10. Statistical analyses

Data from the different measurements were subjected to one-way analysis of variance; comparison of means was performed using a least-square difference (LSD) method. A confidence interval at the 95% level ($p < 0.05$) was considered in all cases.

3. Results and discussion

3.1. Composition analyses

For all kinds of canned products, salmon muscle had lost part of its original colour intensity and showed low tissue integrity. In addition, some water dripping could be observed, leading to some muscle shrinkage as a result of the canning process.

The moisture and lipid contents of canned salmon ranged from 665.0 to 688.0 and 25.0 to 53.0 g kg^{-1} wet muscle, respectively (Table 1). Values for both constituents did not provide significant differences ($p > 0.05$) as a result of the preliminary icing time. Differences in mean values for lipid content can be attributed to fish-to-fish variation.

Compared to the raw fish initial value, all kinds of canned samples showed lower ($p < 0.05$) moisture contents, according to previous research (Castrillón, Álvarez-Pontes, García, & Navarro, 1996; García-Arias, Sánchez-Muniz, Castrillón, & Navarro, 1994). Water loss can be explained in terms of heat treatment and protein degradation in fish muscle, this leading to a decreasing water holding capacity of the myofibrillar protein fraction. For lipid content in muscle, an important increase ($p < 0.05$) is found for all kinds of canned samples when compared to the raw fish value. This relative increase can be explained by the muscle water loss and by the coating oil absorption into the fish muscle during the sterilisation step and the canned storage (Castrillón et al., 1996; García-Arias et al., 1994).

NaCl content (Table 1) showed a great increase ($p < 0.05$) in canned fish when compared to the initial raw fish. This can be explained partly by the NaCl addition during the manufacturing of the canned product before the sterilisation step is achieved, so that part of it might have been absorbed by the fish muscle. On the other hand, chilled fish was in contact with slurry ice, which includes the presence of NaCl; thus, part of the resulting NaCl increase observed in the canned product may be produced by absorption of NaCl into the muscle during the chilled storage. In this sense, previous research (Losada et al., 2005) has shown a NaCl content increase in chilled fish muscle treated under slurry ice conditions as a result of increasing the storage time. According to this, a higher NaCl content was found in the present experiment for canned salmon that had been chilled the longest time (9 days) when compared to the canned samples corresponding to 0 and 5 days of chilled storage.

3.2. Nucleotide degradation

During postmortem fish storage, nucleotides in the muscle tissue degrade in a series of stages as a result of endogenous biochemical changes. The level of major adenine nucleotides and their related compounds (*K* value assessment) have been utilised extensively as an index of freshness of fish muscle before bacterial spoilage commences (Olafsdóttir et al., 1997). It has been documented (Hughes & Jones, 1966; Vázquez-Ortiz, Pacheco-Aguilar, Lugo-Sánchez, & Villegas-Ozuna, 1997) that nucleotides are fairly stable during heating in the canning process and subsequent canned storage, so that nucleotide degradation analysis could be carried out in canned fish to define the degree of fish freshness before processing.

Table 1
Assessment of composition parameters[A] in canned salmon muscle that was preliminary chilled[B]

Canned muscle sample	Moisture content (g kg^{-1} muscle)	Lipid content (g kg^{-1} muscle)	NaCl content (g kg^{-1} muscle)
Starting fish (day 0)	672.0 (8.4)	43.9 (7.7)	10.0[a] (0.2)
Chilled fish (5 days)	672.7 (5.4)	30.6 (6.2)	10.3[a] (2.7)
Chilled fish (9 days)	682.3 (5.2)	36.7 (14.3)	14.4[b] (0.3)

[A] Mean values of four independent determinations ($n = 4$). Standard deviations are indicated in brackets. For each parameter, mean values followed by different letters (a, b) indicate significant ($p < 0.05$) differences as a result of the previous chilling time.
[B] Initial raw fish values: 756.6 ± 15.7 (moisture), 24.6 ± 10.3 (lipids), 0.5 ± 0.1 (NaCl).

In this sense, the presence of the different molecules resulting from the ATP degradation was studied in the different samples of the experiment. According to the previous knowledge, the present study revealed a rapid degradation of ATP into IMP after slaughtering that was accompanied by INO and HX formation. The change of these three molecule contents was studied (Fig. 1).

IMP content did not provide a significant decrease ($p > 0.05$) in the canned samples as a result of increasing the previous chilling time. IMP has been recognized 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 fish. INO and HX contents (Fig. 1) provided a progressive increase ($p < 0.05$) by increasing the previous chilling time. Degradation of IMP to INO and HX has been reported to be catalysed mainly by endogenous IMP phosphohydrolase and INO ribohydrolase, with a contribution from bacterial enzymes as storage time increases (Howgate, 2005).

According 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 the ATP degradation, and accordingly, the freshness loss (Olafsdóttir et al., 1997). In the present experiment, the *K* value analysis (Table 2) provided a progressive and regular increase in canned fish as a result of increasing the previous chilling time. This result agrees to previous research (Hughes & Jones, 1966; Vázquez-Ortiz et al., 1997) supporting the possibility that the *K* value assessment could provide an indication concerning the freshness quality of the raw material employed for canning. Thus, no effect ($p > 0.05$) was observed for the thermal treatment when comparing the initial raw fish values and those corresponding to canned fish from day 0 (Table 2).

However, present *K* values for canned salmon were found largely lower than those reported for coho salmon (Aubourg et al., 2007) and other salmonid species (Erikson, Beyer, & Sigholt, 1997), when considering chilled fish for a similar lasting time (up to 9 days) under traditional flake ice conditions. As a partial explanation, it could be argued that slurry ice system has shown to partially inhibit autolysis when compared to traditional flake ice conditions (Losada et al., 2005). However, more research should be carried out to assess the effect of thermal treatment on the *K* value. In the present case, the *K* value of chilled fish (5 and 9 days) was not measured and could not be compared to its counterpart canned fish.

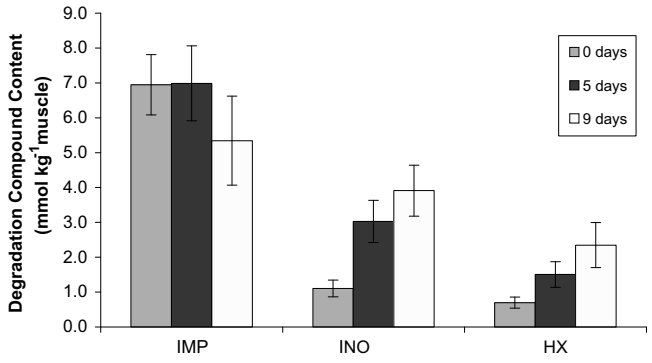

Fig. 1. Change of inosine 5′-monophosphate (IMP), inosine (INO) and hypoxanthine (HX) contents in canned salmon from day 0 and after a chilled storage (5 and 9 days). Bars denote standard deviation of the mean ($n = 4$).

3.3. Volatile amine formation and pH evolution

The trimethylamine (TMA) formation (Table 2) did not provide significant differences ($p > 0.05$) as an effect of the previous chilling time. TMA presence in the canned samples can be explained by means of two different pathways: (i) As a result of trimethylamine oxide (TMAO) bacterial catalysis breakdown during the chilled storage, and (ii) TMA can be produced from TMAO by thermal breakdown during the cooking and sterilisation steps. The great differences found between the raw fish TMA-N values and those from canned fish from day 0, lead to the conclusion that both thermal steps (cooking and sterilisation) have exerted a higher effect on the TMA formation than the previous chilled storage. Previous research on fish canning has already shown a great TMA formation as a result of thermal treatments (Gallardo, Pérez-Martín, Franco, Aubourg, & Sotelo, 1990).

The amine formation was also measured as total volatile bases (TVB; Table 2). The TVB-N content quantifies a wide range of basic volatile compounds (NH_3, methylamine, dimethylamine, trimethylamine, etc.). As in the case of TMA assessment, the TVB-N content measurement showed a great increase in canned fish as a result of the thermal process, according to previous research on tuna fish (Gallardo et al., 1990; Gill, Thompson, Gould, & Sherwood, 1987). A significant effect ($p < 0.05$) of the previous chilling time was observed in the sense that a higher TVB-N content was obtained in canned salmon previously stored during 5 and 9 days than in their counterpart canned fish from day 0.

The pH value has been employed often as a complementary analysis to fish spoilage. In the present case, the pH value (Table 2) did not provide significant differences ($p > 0.05$) as a result of the previous chilled storage time. As an explanation, it could be argued that a long chilled time was not used in the present study, so that an important amine formation during the chilled storage was not likely to be produced. No differences ($p > 0.05$) were also obtained as a result of the thermal process, according to the comparison between raw fish value and canned fish from day 0.

3.4. Lipid damage analysis

A progressive FFA content increase as a result of increasing the previous chilling time could be outlined in the canned samples (Table 3). Comparison between the initial raw fish values and those corresponding to canned fish from day 0 showed that the thermal treatment has also led to an important FFA content increase ($p < 0.05$).

Different mechanisms involved in lipid hydrolysis can be discussed in the light of the different steps considered in this study. According to the relatively short chilled time presently studied,

Table 2
Assessment of autolysis and spoilage parameters[A] in canned salmon muscle that was preliminary chilled[B]

Preliminary chilling time (days)	*K* value (%)	TMA-N (mg kg^{-1} muscle)	TVB-N (mg kg^{-1} muscle)	pH
Starting fish (day 0)	8.7^{a} (0.5)	35.6 (5.1)	362.2^{a} (61.1)	6.7 (0.02)
Chilled fish (5 days)	17.3^{b} (2.7)	36.8 (3.2)	460.3^{b} (20.8)	6.6 (0.03)
Chilled fish (9 days)	24.1^{c} (2.1)	35.5 (1.9)	452.8^{b} (11.9)	6.6 (0.04)

[A] Mean values of four independent determinations ($n = 4$). Standard deviations are indicated in brackets. For each parameter, mean values followed by different letters (a, b, c) indicate significant ($p < 0.05$) differences as a result of the previous chilling time. Abbreviations employed: TMA-N (trimethylamine-nitrogen) and TVB-N (total volatile base-nitrogen).
[B] Initial raw fish values: 7.3 ± 0.7 (*K* value), 0.5 ± 0.1 (TMA-N), 232.8 ± 6.7 (TVB-N), and 6.6 ± 0.04 (pH).

Table 3
Assessment of different lipid damage indices[A] in canned salmon muscle that was preliminary chilled[B]

Preliminary chilling time (days)	FFA (g kg^{-1} lipids)	PV (meq active oxygen kg^{-1} lipids)	AV	TBA-i (mg malondialdehyde kg^{-1} muscle)	PI
Starting fish (day 0)	3.3^{a} (0.4)	1.5 (0.6)	28.8^{b} (0.1)	1.5 (0.2)	1.8 (0.1)
Chilled fish (5 days)	6.3^{b} (0.7)	1.4 (0.3)	29.0^{b} (5.4)	1.8 (0.3)	1.9 (0.2)
Chilled fish (9 days)	8.2^{b} (1.6)	1.9 (0.4)	13.3^{a} (1.1)	1.9 (0.3)	1.7 (0.3)

[A] Mean values of four independent determinations ($n = 4$). Standard deviations are indicated in brackets. For each index, mean values followed by different letters (a, b) indicate significant ($p < 0.05$) differences as a result of the previous chilling time. Abbreviations employed: FFA (free fatty acids), PV (peroxide value), AV (anisidine value), TBA-i (thiobarbituric acid index), and PI (polyene index).
[B] Initial raw fish values: 1.6 ± 0.7 (FFA), 1.4 ± 0.6 (PV), 1.2 ± 0.3 (AV), 0.02 ± 0.01 (TBA-i), and 2.1 ± 0.2 (PI).

FFA formation would be produced by catalysis of endogenous enzymes, since the microbial effect is known to be significant only after the end of the lag phase (Olafsdóttir et al., 1997). Further, during the thermal treatment (cooking and sterilisation), breakdown of high-molecular weight (triglycerides and phospholipids, namely) lipids would be likely to occur and be the source of new FFA formation (Aubourg, Gallardo, & Medina, 1997; Medina, Sacchi, & Aubourg, 1994).

Lipid oxidation was measured by assessment of primary (peroxide value) and secondary (TBA-i and AV) oxidation compounds. The PV (Table 3) did not demonstrate differences ($p > 0.05$) as a result of thermal treatment and the previous chilling time. In all cases, low values were obtained. It could be argued that during the canning process, an important thermal breakdown of primary oxidation compounds is likely to occur so that their assessment would not allow an accurate tool for assessing lipid damage progress (Aubourg & Medina, 1997).

The anisidine value showed an important effect of the previous chilling time. Thus, samples corresponding to fish chilled for 9 days provided a content decrease when compared to the remaining canned samples. Molecules susceptible to be measured under this index (alpha-unsaturated aldehydes, namely) are known to be more reactive than saturated aldehydes, according to a Michael-type reaction, consisting of a beta attack by a nucleophile compound (McMurry, 1993). As a result of an increased damage in canned fish corresponding to 9 days of chilled storage, a higher nucleophile compound content would be expected to exist, so that the mentioned Michael attack would be more likely to occur and lead to an alpha-unsaturated aldehyde content decrease.

In the case of thiobarbituric acid reactive substance (TBARS) content (Table 3), higher mean values are observed when increasing the previous chilling storage time. However, differences were not significant ($p > 0.05$), so that this index was not found useful in order to assess the effect of the previous chilling time. In this sense, it could be argued that TBARS may be partially lost during thermal treatment as a result of interaction with nucleophilic compounds or be leached into the liquid phase of the can (Aubourg & Medina, 1997; Aubourg et al., 1997). For both AV and TBARS indices, comparison of the initial raw fish values with those of canned fish from day 0 showed an important secondary lipid oxidation formation ($p < 0.05$) as a result of the thermal process.

Finally, the fatty acid composition was analysed in the raw and canned fish samples. The PI assessment (Table 3) did not provide differences ($p > 0.05$) in canned fish as a result of the previous chilling time. However, a PI decrease ($p < 0.05$) could be outlined as a result of the thermal treatment. Since previous canning research has shown that the PUFA proportion in total lipids did not diminish as a result of thermal process (Aubourg & Medina, 1997), the presently obtained decrease could be mostly explained by a partial absorbance of palmitic fatty acid (C16:0) from the coating oil employed into the salmon muscle.

3.5. Interaction compound formation

The fluorescent compound assessment was achieved on the coating oil and on the aqueous phase resulting from the lipid extraction of the fish muscle (Table 4). According to the muscle study, a significant ($p < 0.05$) fluorescence formation could be concluded as a result of the thermal treatment and also by effect of an increasing chilling time.

Concerning the coating oil analysis (Table 4), the sterilisation and canned storage steps led to a significant ($p < 0.05$) fluorescence

Table 4
Assessment of interaction compound formation[A] in canned salmon (fish muscle and coating oil) that was preliminary chilled[B]

Canned sample	FR (fish muscle)	FR (coating oil)	Browning development (fish muscle)	Browning development (coating oil)
Starting fish (day 0)	1.17^{a} (0.14)	1.61^{a} (0.11)	1.13^{a} (0.11)	2.14^{a} (0.37)
Chilled fish (5 days)	1.55^{ab} (0.32)	1.75^{a} (0.13)	1.44^{ab} (0.41)	2.21^{a} (0.13)
Chilled fish (9 days)	1.72^{b} (0.22)	2.48^{b} (0.38)	1.72^{b} (0.23)	3.14^{b} (0.25)

[A] Mean values of four independent determinations ($n = 4$). Standard deviations are indicated in brackets. For each index, mean values followed by different letters (a, b) indicate significant ($p < 0.05$) differences as a result of the previous chilling time. Abbreviations employed: FR (fluorescence ratio).
[B] Initial raw fish values: 0.84 ± 0.14 (FR), and 0.87 ± 0.11 (browning). Initial oil values: 1.16 ± 0.01 (FR), and 0.86 ± 0.10 (browning). Values for oil canned in the absence of fish muscle: 1.54 ± 0.04 (FR), and 1.02 ± 0.23 (browning).

formation, since a lower FR value was obtained in the initial oil than in oils canned in the presence or absence of fish muscle. A relevant effect of the previous chilling time could also be assessed, in the sense that the longest previous chilling time led to the highest fluorescence value in canned oil. The presence of the fish muscle did not provide a significant difference ($p > 0.05$) in the FR value when comparing canned oil in the absence of fish muscle and oil that was canned in the presence of fish muscle from day 0; however, if longer chilling times are considered, a higher FR ($p < 0.05$) is obtained for the corresponding oil ones.

Browning development was measured in the lipid extract of the fish muscle and in the coating oil (Table 4). The fish muscle analysis showed similar conclusions than in the case of the FR study. Thus, an increasing ($p < 0.05$) browning development could be outlined as a result of thermal treatment, and also by increasing the previous chilling time.

Concerning the coating oil analysis, a relevant effect of the previous chilling time was observed, so that the highest browning development was observed in oil canned with fish muscle that was previously chilled for 9 days. This result can be explained on the basis of an increasing primary and secondary lipid oxidation development during the previous chilling time; thermal treatment would then favour the reaction of such oxidation compounds with nucleophilic molecules present in the fish muscle to produce browning. Finally, the coating oil would be able to partially extract such interaction compounds supporting browning and fluorescence properties. Related to this, an important content decrease of secondary lipid oxidation compounds (AV) was observed in canned samples that had been kept during 9 days under chilled conditions.

Oil sterilisation in the presence of fish muscle showed a higher browning development ($p < 0.05$) than in the initial oil and in oil canned in the absence of fish muscle. According to the fluorescence analysis, in the absence of fish muscle a browning development is not observed ($p > 0.05$) in canned oil.

Results obtained for interaction compound formation (fluorescence and browning developments) in both fish muscle and coating oil show an important effect of the thermal process and the previous chilling time on chemical changes related to quality loss (Aubourg & Medina, 1997; Aubourg et al., 1992). Interaction compound formation has been reported to be responsible for several detrimental effects during canning (loss of essential nutrients, formation of undesirable compounds, browning development and lipid and protein damage) that can strongly influence the sensory and nutritional quality of canned fish products (Aubourg et al., 1992; García-Arias et al., 1994).

Increasing interaction compound formation agrees with the above mentioned results on primary and secondary lipid oxidation compounds. Thus, degradation products that are susceptible to be measured in primary and secondary oxidation indices can either be distributed into different phases of the can (packing medium, exudates, fish muscle), be partially destroyed during the heat process, or interact with other constituents, so that their determination cannot always allow an accurate method for the quality assessment (Aubourg & Medina, 1997; Aubourg et al., 1992).

3.6. Sensory analysis

The rancid odour development was assessed in the canned fish muscle and in the coating oil. Results are shown in Fig. 2. For both kinds of samples, low scores obtained indicate a low development of rancidity, so that canned products can be considered as greatly accepted. No effect ($p > 0.05$) of the previous chilling time could be assessed. Putrid odour formation was also tested; however, no appreciable putrid odour development was detected in any of the canned samples (fish muscle and coating oil).

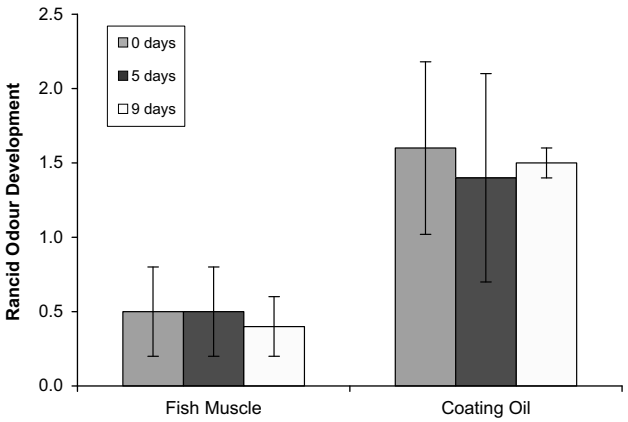

Fig. 2. Rancid odour development in canned salmon (fish muscle and coating oil) from day 0 and after a chilled storage (5 and 9 days). Bars denote standard deviation of the mean ($n = 4$).

Higher rancid odour scores were obtained for coating oils than for their counterpart muscles. This result can be explained by the ability of the coating oils to extract from canned muscle the resulting small and volatile molecules related to rancidity development and accordingly, rancid odour. Values were, however, largely acceptable in all cases.

In the present experiment, sensory analysis was not found useful in order to differentiate canned samples quality by effect of their previous chilling time. A different conclusion is found for chemical indices such as *K* value, TVB-N, FFA, AV and interaction compound formation where, some differences could be assessed as a result of the previous chilling time.

Among the different lipid damage parameters studied in the present experiment, secondary lipid oxidation compounds are known to be the most closely related to the formation of oxidised flavours (White, 1994). In the present study, rancid odour evolution showed a better agreement with TBARS assessment than with the AV detection.

4. Conclusions

The different steps included in the canning processing have led to important chemical changes during the preparation of farmed coho salmon as a canned product. First, an increasing previous chilling time has led to an important autolysis (*K* value) development, and to an increasing formation of free fatty acids, and interaction compounds (fluorescence and browning assessments) in the canned product ($p < 0.05$). Further, the thermal treatment (cooking and sterilisation) has led to the formation of volatile amines (total and trimethylamine), free fatty acids, secondary lipid oxidation compounds (anisidine and thiobarbituric acid values) and interaction compounds in the canned fish.

Interaction compound assessment was found the most useful tool to study the lipid oxidation and non-enzymatic browning developments. The *K* value has shown a progressive and regular increase in canned fish as a result of increasing the previous chilling time. Further research focused onto the employment of such index to assess the freshness stage of the raw material employed is considered promising.

In spite of all the chemical changes observed, sensory assessment of the canned product showed that good quality products were obtained even when considering the canned fish corresponding to the longest previous chilling time. Employment of farmed fish species, where slaughtering and previous storage conditions can be conveniently mastered, is greatly recommended for canning purposes.

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