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**Changes in the flesh of cooked farmed salmon
(*Oncorhynchus kisutch*) with previous storage in slurry
ice (-1.5°C)**

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1 **ABSTRACT**

2 Whole, farmed Coho salmon (*Oncorhynchus kisutch*) were sacrificed in slurry
3 ice (-1.5°C) then stored in this medium for further processing after 0, 5 and 9 days. They
4 were cooked whole and the flesh was evaluated by sensory, physical and chemical
5 techniques to establish if significant changes had occurred as a result of the storage
6 period. Initial samples from harvest were also evaluated for comparison. There was
7 evidence of increases in trimethylamine, lipid hydrolysis, lipid oxidation (anisidine and
8 thiobarbituric acid values) and interaction compound formation (fluorescence and
9 browning measurements). The fish structure became more breakable with longer storage
10 but there were no changes in sensory assessments for rancid and putrid odours, so that
11 scores were less than 0.5 on a 11 point scale. From the present results, primary and
12 secondary lipid oxidation development and further interaction compound formation
13 appears to be the main measurable indicators of quality changes in cooked Coho
14 salmon. However, and according to sensory appreciation, slurry ice has shown to be a
15 suitable medium for previous storage of Coho salmon for periods of up to 9 days.

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19 **Key Words:** *Oncorhynchus kisutch*, farming, chilling, cooking, deterioration, quality

20 **Running Title:** Quality deterioration in cooked farmed salmon

INTRODUCTION

1
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3 Cooking destroys pathogenic and spoilage microorganisms, inactivates enzymes
4 and enhances desirable flavours and tastes of fish flesh (McLay, 1982). However, owing
5 to the thermal sensitivity of a broad number of fish constituents and nutrients several
6 detrimental effects due to cooking have been reported: i.e., heat degradation of
7 nutrients, oxidation of vitamins and lipids, leaching of water-soluble vitamins, minerals
8 and proteins, and toughening and drying of sensitive protein tissues (Aitken & Connell,
9 1979; Pigott & Tucker, 1990).

10 Most of the quality problems found in cooked fish products are directly related
11 to the initial quality of the fresh raw material, which declines continuously post-mortem
12 during its preliminary refrigerated storage (Olafsdóttir et al., 1997). Accordingly,
13 quality of processed fish will depend to a large extent on the adequacy of the
14 preliminary holding methods used (Slabyj & True, 1978; Aubourg & Medina, 1997). In
15 this sense, great efforts have been carried out in the search of appropriate chilling
16 conditions so that autolytic degradation and microbial spoilage would be slowed and
17 minimised (Whittle, Hardy, & Hobbs, 1990; Ashie, Smith, & Simpson, 1996).

18 In recent years the fishing sector has suffered from dwindling stocks of
19 traditional species as a result of dramatic changes in their availability. This has
20 prompted fish technologists and the fish trade to pay more attention to aquaculture
21 techniques as a source of fish and other seafood products (Stickney, 1990). Among
22 cultivated fish, Coho salmon (*Oncorhynchus kisutch*), also called silver salmon, has
23 received great attention because of its increasing production in countries like Chile,
24 Japan and Canada (FAO, 2006a) in parallel to important capture production in countries
25 such as USA, Russian Federation, Canada and Japan (FAO, 2006b). Most research on

1 this fish species has been carried out on genetic aspects and farming conditions during
2 aquaculture production (Estay, Díaz, Neira, & García, 1997; Winkler, Bartley, & Díaz,
3 1999). However, previous research concerning quality changes produced during
4 processing has been scarce, only accounting for freezing (Braddock & Dugan, 1972;
5 Rodríguez, Losada, Larraín, Quitral, Vinagre, & Aubourg, 2007) and chilling (Barnett,
6 Nelson, & Poysky, 1991; Aubourg, Quitral, Larraín, Rodríguez, Gómez, Maier, &
7 Vinagre, 2007) conditions.

8 The present study focuses on the employment of farmed fish species as raw
9 material for the commercialisation of thermally-treated fish products. In it, Coho salmon
10 was chosen, so that the effect of a short preliminary chilled storage on quality
11 degradation in the resulting cooked product was studied through sensory, physical and
12 chemical changes. Because temperature control is so important, an advanced biphasic
13 chilling system in which slurry ice holds the fish at a lower temperature than traditional
14 flake ice was employed as slaughtering and holding process (Yamada, Fukusako, &
15 Kawanami, 2002).

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18 **MATERIALS AND METHODS**

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20 **Chilling system (slurry ice) elaboration**

21 A slurry ice prototype (FLO-ICE, Kinarca S.A.U., Vigo, Spain) was used. The
22 composition of the slurry ice binary mixture was 40 % ice/60 % water, prepared from
23 filtered seawater (salinity: 3.3 %). The temperature of the slurry ice mixture was $-1.5\text{ }^{\circ}\text{C}$
24 and the calculated salt content about 2.0%. The average temperature of the specimens
25 was in the range of $-1.0\text{ }^{\circ}\text{C}$ to $-1.5\text{ }^{\circ}\text{C}$.

Raw fish, chilling storage, sampling, cooking and chemicals

Specimens of farmed Coho salmon (*Oncorhynchus kisutch*) (weight range: 2.8-3.2 kg) were obtained from *Comercial Xanquéi* (Lousame, La Coruña, Spain) in May 2006. Individual fish gonads were at the 4th/5th stage of Maier's scale of gonad maturity. The fish were sacrificed at the cultivation plant by immersion in slurry ice. Individuals were kept under this chilling condition during transportation to the laboratory. Upon arrival in the laboratory, 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. The slurry ice mixture was renewed each three days of storage. During the chilled storage, once in a day a temperature logger was employed at different parts of the fish to monitor its temperature.

Twenty-four hours after slaughtering, four individuals were not thermally-treated and were studied as initial raw fish (day 0). The remaining fish (12 individuals) were taken for the cooking process on days 0, 5, and 9 of chilled storage. Whole and ungutted salmon specimens were steam cooked during 25 minutes 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 hours. For each individual fish, the white muscle was then collected and splitted into three different parts. Two of them were directly employed for the sensory and physical analyses, respectively; the third one was homogenised and used for the chemical analyses. Both in raw and in cooked samples, each individual fish was studied separately from others to achieve the statistical study (n=4).

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

1 **Composition analyses**

2 Water content was determined by weight difference between the homogenised
3 fish muscle (1-2 g) before and after 24 h at 105 °C. Results are expressed as g water /
4 100 g muscle.

5 The lipid fraction was extracted from the fish muscle by the Bligh and Dyer
6 (1959) method. Quantification results are expressed as g lipid / 100 g muscle.

7 NaCl contents were determined after boiling portions of fish muscle in the
8 presence of HNO₃, followed by the addition of excess 0.1N AgNO₃ and the titration of
9 non-neutralised silver nitrate with 0.1N NH₄SCN (AOAC, 1990). The results are
10 expressed as g NaCl / 100 g muscle.

11

12 **Volatile amine formation and pH assessment**

13 Total volatile base-nitrogen (TVB-N) values were measured by the
14 Antonacopoulos (1960) method, with some modifications. Briefly, fish muscle (10 g)
15 was extracted with 6% (w/v) perchloric acid and brought up to 50 ml, determining the
16 TVB-N content –after steam-distillation of the acid extracts rendered alkaline to pH 13
17 with 2% (w/v) NaOH – by titration of the distillate with 10 mM HCl. The results are
18 expressed as mg TVB-N / 100 g muscle.

19 Trimethylamine-nitrogen (TMA-N) values were determined by means of the
20 picrate method, as previously described (Tozawa, Erokibara, & Amano, 1971). This
21 involves the preparation of a 5% (w/v) trichloroacetic acid extract of fish muscle. The
22 results are expressed as mg TMA-N / 100 g muscle.

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

25

1 **Lipid damage analysis**

2 Free fatty acid (FFA) content was determined by the Lowry and Tinsley (1976)
3 method based on complex formation with cupric acetate-pyridine followed by
4 spectrophotometric (715 nm) assessment. Results are expressed as g FFA / 100 g lipids.

5 Primary lipid oxidation was determined by means of the peroxide value (PV)
6 according to the ferric thiocyanate method (Chapman and McKay, 1949). The results
7 are expressed as meq active oxygen / kg lipids.

8 The anisidine value (AV) was determined in fish muscle according to the AOCS
9 (1993) method, based on the reaction between α - and β -unsaturated aldehydes
10 (primarily 2-alkenals) and p-anisidine reagent. AV is expressed as 100 times the
11 absorbance measured at 350 nm in a 1 cm path length cuvette from a solution
12 containing 1 g lipid / 100 ml reaction medium.

13 The thiobarbituric acid index (TBA-i) was determined according to Vyncke
14 (1970). This method is based on the reaction between a trichloroacetic extract of the fish
15 muscle, and thiobarbituric acid at high temperature (95-97°C), the resulting
16 chromophore being measured at 532 nm. Results are expressed as mg malondialdehyde
17 / kg fish muscle.

18

19 **Interaction compound formation**

20 Formation of fluorescent compounds was determined with a Perkin Elmer LS 3B
21 fluorimeter by measurements at 393/463 nm and 327/415 nm as previously described
22 (Aubourg & Medina, 1997). The relative fluorescence (RF) was calculated as follows:
23 $RF = F/F_{st}$, where F is the fluorescence measured at each excitation / emission
24 maximum, and F_{st} is the fluorescence intensity of a quinine sulphate solution (1 μ g / ml
25 in 0.05 M H_2SO_4) at the corresponding wavelength. The fluorescence ratio (FR) was

1 calculated as the ratio between the two RF values: $FR = RF_{393/463} \text{ nm} / RF_{327/415} \text{ nm}$. The
2 FR value was determined in the aqueous phase resulting from the lipid extraction of the
3 fish muscle.

4 Browning development was determined spectrophotometrically at 420 nm in the
5 lipid extract of the edible flesh. The results were calculated using the equation:
6 Browning = $A \times V / w$, where A is the absorbance reading at 420 nm, V is the volume
7 (ml) of the sample and w is the amount (mg) of the lipid sample.

8

9 **Textural analysis**

10 A shear test was used to evaluate texture. Firmness and cohesivity were
11 determined from a stress-distance curve obtained from a Universal Testing Machine
12 (Lloyd Instruments Limited, LR-5K, Hampshire, United Kingdom) including a load cell
13 of 500 N (Jonsson, Sigurgisladóttir, Hafsteinsson, & Kristbergsson, 2000). The shear
14 force or firmness was measured as the maximum peak force (N) required to shear/cut
15 through the samples; cohesivity was measured during the upward movement of the
16 blade and was calculated as the deformation (mm) at maximum peak force
17 (Sigurgisladóttir, Hafsteinsson, Jonsson, Nortvedt, Thomasses, & Torrisen, 1999).

18

19 **Sensory analysis**

20 The analysis of rancid and putrid odour development was conducted by a
21 sensory panel consisting of ten experienced judges, according to Howgate (1992).
22 Panellists had been involved in sensory analysis of different kinds of fish foods during
23 the last ten years. Previously to the present experiment, a special training was carried
24 out concerning raw and cooked salmon of different quality conditions.

1 At each sampling time, the fish muscle portions were presented to panellists in
2 individual trays and were scored individually. The panel members shared samples
3 tested. Rancid and putrid odour developments were evaluated using a Quantitative
4 Descriptive Analysis (QDA) on a non-structured linear scale with numerical scores
5 from 0 (stage of no rancidity/ putridity at all) to 10 (stage where no increase in
6 rancidity/ putridity is possible); score 5.0 was considered the borderline of fish
7 acceptability. Scores among panellists were averaged.

8

9 **Statistical analyses**

10 Data from the different measurements were subjected to one-way analysis of
11 variance; comparison of means was performed using a least-square difference (LSD)
12 method (Statsoft, 1994). A confidence interval at the 95% level ($p < 0.05$) was
13 considered in all cases.

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15

16 **RESULTS AND DISCUSSION**

17

18 **Composition analyses**

19 The water and lipid contents of cooked salmon were included in the ranges
20 68.20-73.70 and 1.80-3.10 g / 100 g wet muscle, respectively (Table 1). Values for both
21 constituents did not result in significant differences as a result of the preliminary icing
22 time; differences in mean values may be attributed to fish-to-fish variation. Lipid
23 content of the white muscle showed to be relatively low; as an explanation, it can be
24 argued that fish individuals employed in the present experiment correspond to the year

1 time of lower lipid content (Hardy & Keay, 1972; Roth, Johansen, Suontama, Kiessling,
2 Leknes, Guldberg, & Handeland, 2005).

3 Compared to the raw fish initial value, all kinds of cooked samples showed
4 lower water contents, according to previous research on mackerel (Hearn, Sgoutas,
5 Sgoutas, & Hearn, 1987) and albacore (García-Arias, Sánchez-Muniz, Castrillón, &
6 Navarro, 1994; Castrillón, Álvarez-Pontes, García, & Navarro, 1996). Water loss can be
7 explained in terms of denaturation of sarcoplasmic and myofibrillar proteins and
8 disruption of the muscle structure, this leading to a decreasing water holding capacity of
9 the protein fraction (Seet & Brown, 1983; Castrillón et al., 1996). For muscle lipid
10 content, a cooking effect was not noticeable in the present experiment, although
11 previous research concerning fattier fish species showed a lipid content increase in
12 muscle as a result of cooking (Gallardo, Aubourg, & Pérez-Martín, 1989; García-Arias
13 et al., 1994).

14 Comparison between the NaCl content in fish muscle before and after the
15 cooking treatment did not result in a significant difference. Concerning the effect of the
16 previous holding time, individual salmons that had been chilled the longest time (9
17 days) showed an important increase in NaCl when compared to their corresponding
18 cooked samples previously chilled during 0 and 5 days. Slurry ice contains about 2%
19 salt and the present results agree with previous research that slow absorption of NaCl
20 occurs into the fish during the storage period (Losada, Piñeiro, Barros-Velázquez, &
21 Aubourg, 2005). Despite that, it should be stressed that the NaCl concentrations
22 determined in cooked salmon after 9 days of storage in slurry ice were found to be
23 lower than those described for fish material subjected to refrigeration in seawater
24 (Smith, Hardt, McDonald, & Templeton, 1980).

25

1 **Volatile amine formation**

2 Increasing mean values of TVB-N were observed with longer storage periods
3 (Table 2), but they were not significant; thus, an effect of the previous chilling time was
4 not concluded. Comparison between raw fish before and after cooking did not lead to
5 significant differences, although previous research carried out on albacore (*Thunnus*
6 *alalunga*) showed a considerable TVB-N increase after cooking (Gallardo, Pérez-
7 Martín, Franco, Aubourg, & Sotelo, 1990). The TVB-N content quantifies a wide range
8 of basic volatile compounds (ammonia, methylamine, dimethylamine, trimethylamine,
9 and so on), that should be produced as a result of microbiological activity during the
10 chilling storage or arise from the thermal breakdown of endogenous compounds during
11 cooking. Throughout the present experiment, the TVB-N index did not result in
12 differences in quality deterioration.

13 Amine formation was also measured by the TMA-N content (Table 2). Its
14 detection showed an important influence of the cooking process and the preliminary
15 chilling time, so that both led to important increases in this metabolite. However,
16 trimethylamine (TMA) formation in Coho salmon was low when compared to other fish
17 species (sardine, albacore tuna) under similar conditions (Slabyj & True, 1978; Gallardo
18 et al., 1990) and agrees to low levels found for trimethylamine oxide (TMAO) and
19 TMA in salmon species (Barnett et al. 1991; Dondero, Cisternas, Carvajal, & Simpson,
20 2004). This low TMA formation may be of positive relevance in the sense that this
21 metabolite is one of the main compounds involved in off-odour production in spoiled
22 fish (Gallardo et al., 1990; Olafsdóttir et al., 1997). TMA formation in the actual cooked
23 samples can be explained by means of two different pathways: i) As a result of TMAO
24 bacterial catalysis breakdown during the chilled storage, and ii) TMA can be produced
25 from TMAO by thermal breakdown during the cooking process. The great differences

1 found for the raw fish TMA-N values before and after cooking leads to the probable
2 conclusion that thermal treatment has exerted a higher effect on the TMA formation
3 than the previous chilled storage.

4 The pH value has been employed often as a complementary analysis to fish
5 spoilage detection. In the present case, the pH value (Table 2) did not result in
6 significant differences as a result of the previous chilled storage time, which agrees to
7 the TVB-N content evolution observed and also agrees to the low TMA formation. As
8 an explanation, it could be argued that a long chilled time was not encountered in the
9 present study, since a lag phase round 12 days was previously observed for Coho
10 salmon species under traditional icing (Aubourg et al., 2007). Furthermore the lower
11 temperature of slurry ice of -1.5°C has a dramatic effect on spoilage being fast slower at
12 this temperature than at 0°C (Yamada et al. 2002). In addition, no differences were
13 observed between the pH value of raw fish before and after the cooking process.

14

15 **Lipid hydrolysis analysis**

16 A progressive increase in FFA occurred during storage (Table 2). Comparison of
17 the initial raw fish before and after cooking showed that the thermal process did not lead
18 to a variation in the FFA content of the fish muscle.

19 Different mechanisms involved in lipid hydrolysis can be discussed at the light
20 of the different processing steps considered in this study. On the one hand, FFA
21 formation during a relatively short chilled time occurs due to catalysis by endogenous
22 enzymes, and only microbial effects would be significant after the end of the lag phase
23 (Whittle et al., 1990; Olafsdóttir et al., 1997). Further, during a thermal treatment,
24 breakdown of high-molecular weight (triglycerides and phospholipids, namely) lipids
25 would be likely to occur and be the source of new FFA formation (Gallardo et al., 1989;

1 Yamamoto & Imose, 1989). The results shown in Table 2 indicate that it is probable
2 that enzymatic formation of FFA is more important than effects of heating.

3 The formation of FFA itself does not lead to nutritional losses. However,
4 accumulation of FFA has been related to some extent to lack of acceptability, because
5 FFA are known to have detrimental effects on protein solubility and cause texture
6 deterioration by interacting with proteins (Sikorwski & Kolakowska, 1994) and oxidise
7 faster than higher-molecular-weight lipid classes (namely, triglycerides and
8 phospholipids) by providing a greater accessibility (lower steric hindrance) to oxygen
9 and other pro-oxidant molecules (Labuza, 1971).

10

11 **Lipid oxidation analysis**

12 The PV (Table 3) detection showed an important peroxide formation as a result
13 of the cooking process, and also as a consequence of increasing the previous chilling
14 time from 0 days to 5 days. Indeed, a remarkable peroxide content was obtained for
15 cooked samples corresponding to a 5 days-chilled period. However, if the chilling time
16 is further increased to 9 days, a peroxide formation drop is obtained in the resulting
17 cooked salmon muscle. As an explanation, it could be argued that once the peroxide
18 formation is initiated, values obtained are a balance between the rates of formation and
19 rates of reaction (Aubourg, 1999).

20 The AV showed an important effect of the cooking process and also of the
21 previous chilling time (Table 3). Thus, both process steps led to an increased formation
22 of molecules susceptible to be measured by this index (alpha-unsaturated aldehydes,
23 namely). Increases can be considered important, so that the AV has shown to be a useful
24 tool for indicating both storage and cooking effects.

1 Secondary lipid oxidation compounds were also measured by the TBA-i (Table
2 3). Similar conclusions about its use in this context were drawn as for AV. Again, the
3 secondary oxidation compound formation resulted in an interesting tool to assess the
4 chemical changes produced as a result of the cooking process and as a result of a
5 preliminary chilled storage. In this sense, previous research already accounts for
6 carbonyl formation during cooking in sardine and tuna fishes (Yamamoto & Imose,
7 1989; Aubourg, Medina, & Pérez-Martín, 1995).

8 9 **Interaction compound formation**

10 Interaction compound formation was measured by means of the fluorescence
11 and browning developments in the salmon muscle extracts, according to the Material
12 and Methods section (Table 3). Both parameters showed a significant formation as a
13 result of cooking and also with longer storage period. This increasing interaction
14 compound formation agrees with the above mentioned results on secondary lipid
15 oxidation compound (AV and TBA-i) and also with the peroxide content decrease
16 observed for the cooked samples where a more advanced deterioration is expected to
17 occur (9 days of preliminary chilling time). These results agree with previous work
18 done on cooking of two tuna fish species (Aubourg et al., 1995).

19 The formation of interaction compounds (Pokorný, 1981; Aubourg, 1999) also
20 called tertiary oxidation compounds, is the result of the interaction between lipid
21 oxidation products (primary and secondary) and protein-like molecules present in fish
22 muscle. The electrophilic character of most lipid oxidation compounds leads them to
23 interact with food constituents possessing nucleophilic functions. Such interaction is
24 highly favoured by a temperature increase. The interactions are very important
25 secondary reactions of oxidised lipids, particularly in protein-rich foodstuffs such as

1 marine sources, which have high proportion of essential and reactive amino acids such
2 as lysine and methionine. As a result, previous research has pointed out a partial loss of
3 polyunsaturated fatty acids and essential amino acids, and some activity decrease of
4 hydrolytic enzymes during cooking (Seet & Brown, 1983; García-Arias et al., 1994;
5 Castrillón et al., 1996).

6

7 **Textural properties**

8 Firmness and cohesivity results are indicated in Figure 1. Firmness resulted in a
9 differential effect of preliminary chilling storage so that an increasing value ($p<0.05$)
10 was obtained with chilling time. On the other hand, cohesivity score showed to decrease
11 with the storage time ($p<0.05$). As a result, it is concluded that a more breakable
12 structure was obtained by enlarging the preliminary chilling storage.

13 The texture of the fish muscle depends on numerous intrinsic biological factors
14 related to the density of the muscle fibres, as fat and collagen content of the fish
15 (Sigurgisladóttir et al., 1999; Olafsdóttir et al., 2004). Previous research has shown that
16 fish become less firm with longer chilling periods (Alasalvar, Taylor, Öksüz,
17 Garthwaite, Alexis, & Grigorakis, 2001). However, heating converts the translucent,
18 jelly-like cellular fish mass into an opaque and firmer material, where the connective
19 tissue holding the cells together has been denatured and blocks of cells become readily
20 separated from one another into flakes (Aitken & Connell, 1979). Changes of texture
21 have been reported to be mostly due to crosslinking of peptide chains by reaction with
22 lipid oxidation products, such as aldehydes (Pokorný, 1981; Sikorski & Kolakowska,
23 1994).

1 **Sensory analysis**

2 The rancid and putrid odour development was assessed in the cooked fish
3 muscle. Results are shown in Figure 2. For both attributes, low scores obtained indicate
4 a low rancid and putrid development, so that cooked fish can be considered as greatly
5 accepted. No effect ($p>0.05$) of the previous chilling time could be assessed, according
6 to the extended shelf life reported for salmon species under chilling conditions
7 (Sveinsdóttir, Martinsdóttir, Hyldig, Jørgensen, & Kristbergsson, 2002).

8 Among the different chemical parameters related to quality loss studied in the
9 present experiment, amine formation (total and TMA) and secondary lipid oxidation
10 compounds (AV and TBA-i) are known to be the most closely related to the formation
11 of putrid and oxidised flavours, respectively (White, 1994; Olafsdóttir et al., 1997).
12 Actual sensory scores on putrid odour development greatly agreed to the results
13 obtained for volatile amine formation (Table 2). However, sensory scores on rancid
14 odour development do not agree to secondary lipid oxidation values (AV and TBA-i)
15 obtained for the different kinds of cooked samples. It is likely that an extended study
16 considering longer previous chilling times would have given more information on the
17 correlation of lipid oxidation and sensory descriptors.

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9 **Acknowledgments**

10 The authors thank *Mr. Marcos Trigo* for technical assistance, *Comercial*
11 *Xanquéi* (Lousame, La Coruña, Spain) for providing the salmon fish and *Kinarca S. A.*
12 *U.* (Vigo, Spain) for lending the slurry ice equipment. The work was realised in the
13 frame of the Chilean University (Chile)-Consejo Superior de Investigaciones Científicas
14 (Spain) Program (Project 2006 CL 0034) and founded by the *Secretaría Xeral de I+D*
15 from the *Xunta de Galicia* (Galicia, Spain) (*Project PGIDIT05TAL00701CT*).

1 **FIGURE LEGENDS**

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6 **Figure 1**: Textural (firmness and cohesivity) change detection in cooked salmon that
7 was preliminary chilled during 0, 5, and 9 days* (■ , ■ , □ , respectively).
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9 * Bars denote standard deviation of the mean (n=4).
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14 **Figure 2**: Odour (rancid and putrid) detection in cooked salmon that was preliminary
15 chilled during 0, 5, and 9 days* (■ , ■ , □ , respectively).
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17 * Bars denote standard deviation of the mean (n=4).
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20
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TABLE 1

Assessment of composition parameters* in raw and cooked salmon that was preliminary chilled

Salmon sample (raw / cooked)	Water content (g/ 100g muscle)	Lipid content (g/ 100g muscle)	NaCl content (g/ 100g muscle)
Raw salmon	75.66 b (1.27)	2.46 (1.03)	0.07 a (0.01)
Cooked salmon (0 days previous chilling)	70.37 a (2.11)	2.69 (0.41)	0.10 ab (0.04)
Cooked salmon (5 days previous chilling)	72.66 a (0.94)	2.42 (0.49)	0.13 b (0.01)
Cooked salmon (9 days previous chilling)	71.81 a (1.75)	2.31 (0.69)	0.21 c (0.01)

* 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 between the different kinds of samples.

TABLE 2

Assessment of spoilage parameters and lipid hydrolysis* in raw and cooked salmon that was preliminary chilled**

Salmon sample (raw / cooked)	TVB-N (mg/ 100g muscle)	TMA-N (mg/ 100g muscle)	pH	FFA (g/ 100g lipids)
Raw salmon	23.28 (0.67)	0.05 a (0.01)	6.61 (0.04)	0.16 a (0.07)
Cooked salmon (0 days previous chilling)	23.21 (0.69)	0.29 b (0.08)	6.61 (0.04)	0.13 a (0.03)
Cooked salmon (5 days previous chilling)	24.87 (0.90)	0.37 bc (0.05)	6.62 (0.02)	0.83 b (0.30)
Cooked salmon (9 days previous chilling)	25.80 (2.75)	0.47 c (0.06)	6.66 (0.02)	2.32 c (0.94)

* 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 between the different kinds of samples.

** Abbreviations employed: TVB-N (total volatile base-nitrogen), TMA-N (trimethylamine-nitrogen), and FFA (free fatty acids).

TABLE 3

Assessment of lipid oxidation and interaction compound formation* in raw and cooked salmon that was preliminary chilled**

Salmon sample (raw / cooked)	PV	AV	TBA-i	FR	Browning
Raw salmon	1.38 a (0.58)	1.15 a (0.25)	0.02 a (0.01)	0.14 a (0.05)	0.87 a (0.11)
Cooked salmon (0 days previous chilling)	4.15 b (0.72)	5.57 b (2.16)	0.52 b (0.10)	0.54 b (0.10)	1.32 b (0.11)
Cooked salmon (5 days previous chilling)	13.80 c (2.06)	25.76 c (1.65)	0.67 b (0.18)	0.79 bc (0.19)	2.04 bc (0.44)
Cooked salmon (9 days previous chilling)	5.52 b (0.56)	53.03 d (0.48)	1.11 c (0.16)	0.93 c (0.11)	2.43 c (0.19)

* 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, c, d) indicate significant ($p < 0.05$) differences between the different kinds of samples.

** Abbreviations employed: PV (peroxide value), AV (anisidine value), TBA-i (thiobarbituric acid index), and FR (fluorescence ratio). Units employed: meq active oxygen/ kg lipids (PV) and mg malondialdehyde/ kg muscle (TBA-i).

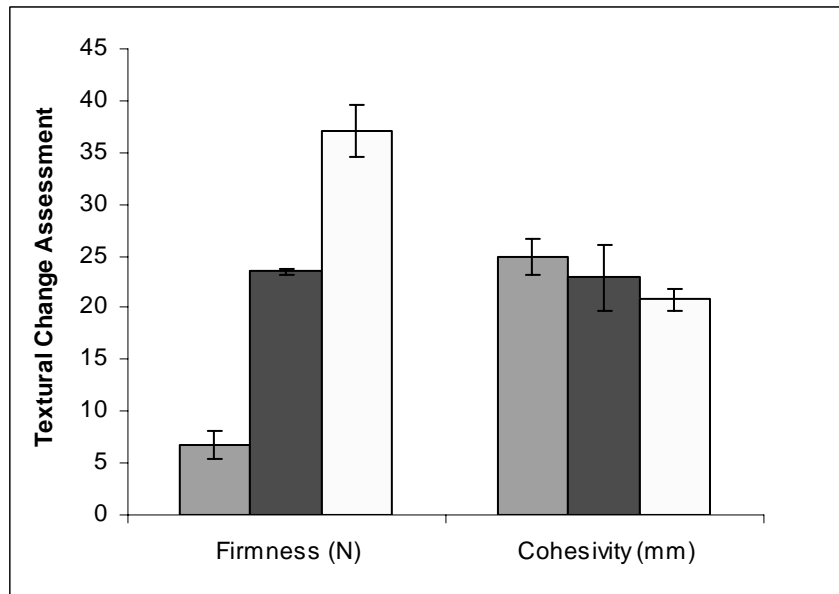


Figure 1

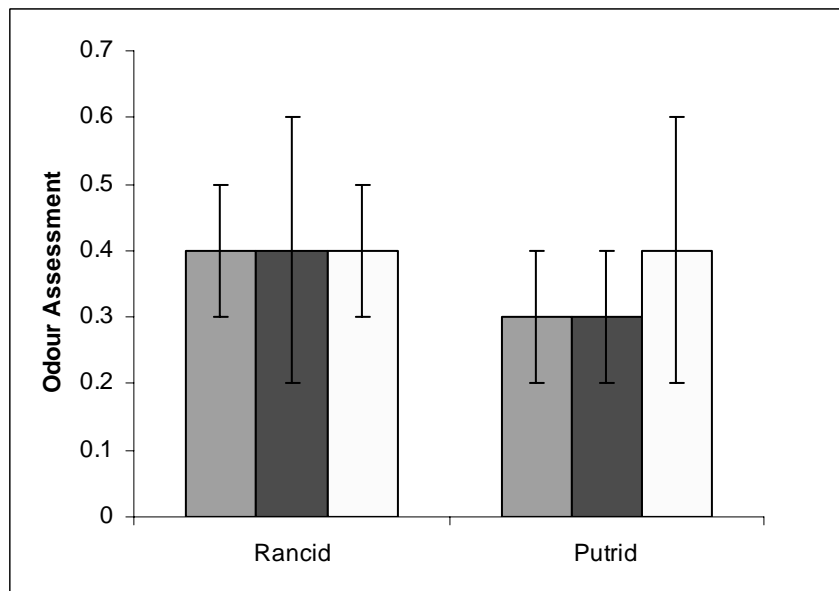


Figure 2