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# Changes in quality of vacuum-packed cold-smoked salmon (*Salmo salar*) as a function of storage temperature

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#### Abstract

Changes in the quality of vacuum-packed cold-smoked salmon (*Salmo salar*) were evaluated through a systematic study of biochemical, microbiological and sensory analyses during storage at different temperatures (0, 2, 4, 6 and 8 °C). TVB, TMA, *K* value, total aerobic and anaerobic counts and *Lactobacillus* spp., showed significant correlation ( $p \le 0.05$ ) with storage time, temperature and sensory quality. Hypoxanthine (Hx), Biogenic amines, molds and yeasts were not considered good objective indicators of sensory quality. Shelf lives of smoked-salmon stored at 0, 2, 4, 6 and 8 °C were 26, 21, 20, 10 and 7 days, respectively. *Lactobacillus* spp., were dominant in terms of deterioration in quality. Pathogenic microorganisms (*Clostridium botulinum, Salmonella*, Coliform, *Staphylococcus aureus* and *Listeria monocytogenes*) were not detected during the time of storage. © 2004 Elsevier Ltd. All rights reserved.

Keywords: Cold-smoked salmon; Vacuum-packed; Fish spoilage; Quality indicators

# 1. Introduction

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In Chile, salmon farming started 30 years ago and today it is a very large and stable industry. The increasing demand, favourable climatic conditions and low cost have led to an exponential growth in this industry and Chile is now the second largest salmon exporter in the world (over 150,000 metric/tonne/year). According to Chilean exported salmon statistics, smoked-salmon have shown a constant and steady increase since 1998. At present, smoked-salmon represents approximately 4% of exported salmon (Salmonchile, 2003). The quality of exported salmon depends mainly on processing conditions and manipulation during distribution. During storage and transportation of the salmon, temperature conditions are often less than ideal and temperature abuses frequently occur. Although the need for temperature controls in the quality and safety of seafood is not a new concern, there is an emerging interest for better controls.

Atlantic salmon (*Salmo salar*) is an important product, from both economic and nutritious perspectives. The maximum shelf life for iced whole salmon is about 20 days and for salmon steaks/fillets under MAP at chilled temperatures (2–4 °C), shelf lives of 14–21 days have been observed (Emborg, Laursen, Rathjen, & Dalgaard, 2002). Smoked-salmon is a ready-to-eat product normally purchased vacuum-packed in slices or pieces. The storage time under refrigeration is often 3–4 weeks, though it may be up to 6 weeks (Rorvik, Yndestad, & Skjerve, 1991).

Storage of vacuum-packed cold-smoked salmon at chilled temperatures results in abundant development of a microflora, but this does not necessarily coincide with the onset of spoilage. No relationship has been found between the total number of microorganisms, sensory quality and shelf life (Gibson & Ogden, 1987; Rorvik et al., 1991).

The role of autolytic enzymes in the loss of quality in cold-smoked salmon is known. During salmon processing, the temperature never exceeds 28 °C, hence there is no heat-inactivation of native enzymes in the tissue (Truelstrup, Gill, & Huss, 1995). Without an objective criterion for quality evaluation, the producers of

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smoked-salmon have difficulties in monitoring changes in quality, and in determining shelf life and they may also be confronted with demands from their customers, which primarily centre on with the microbiological status of the product.

Numerous individual chemical measurements are used as indices of spoilage in cold-smoked salmon, e.g., acetic acid, ethanol, hypoxanthine, trimethylamine and total volatile bases (Truelstrup, Gill, Drewes, & Huss, 1996). A new quality index, based on concentrations of cadaverine, histamine, putrescine, tyramine and pH, showed good correlation with sensory assessments (Jorgensen, Dalgaard, & Huss, 2000).

The aim of this study was to find suitable objective quality indicators through a systematic study of chemical, microbiological and sensory changes during storage of vacuum-packed cold-smoked salmon (*Salmo salar*) at different temperatures  $(0, 2, 4, 6 \text{ and } 8 \text{ }^{\circ}\text{C})$ .

#### 2. Materials and methods

# 2.1. Salmon (Salmo salar)

Cold-smoked salmons were processed at Finamar Co., Puerto Montt, Chile. The fillets when first obtained from the whole fish, had a relative humidity (RH) of 80%. They were then dried for 5 h at 24 °C, to obtain a final RH of 60%. Thereafter, the samples were treated with 2.7% NaCl, 0.05% TBHQ and 0.026% sodium benzoate. They were smoked at 24 °C for 1.5 h at 60% RH. The fish were sliced and vacuum-packed in 200 g portions in bags consisting of 70% polystyrene and 30% polyamide. After packing, samples were immediately stored at 0, 2, 4, 6 and 8 °C. Final salt and fat contents in the smoked fillets were 2.01% and 8.95%, respectively.

#### 2.2. Chemical analysis

Hypoxanthine (Hx), inosine monophosphate (IMP) and inosine (INO) were determined as described by Ryder (1985). The K value was calculated according to Huynch, Mackey, and Gawley (1992). Total volatile bases (TVB) and trimethylamine (TMA) were measured according to Sernapesca (1996). Biogenic amines (histamine, putrescine and cadaverine) were determined by HPLC (Veciana, Hernández, Marine, & Vidal, 1995).

## 2.3. Microbiological analysis

Total viable counts (TVC), aerobic and anaerobic, Enterobacteriaceae, molds, yeasts, lactic acid bacteria, *Staphylococcus aureus*, *Listeria monocytogenes*, *Clostridium botulinum* and *Salmonella*, were determined as described by US Food and Drugs Administration (FDA, 1992).

# 2.4. Sensory evaluation

Sensory evaluation was carried out with 8–10 trained panellists. Samples were evaluated for overall acceptability with regard to odour, flavour, colour and texture. A hedonic scale was used with numerical scores from 1 to 7, where 1 and 7 were considered the lowest and highest, respectively, and 4 was considered the borderline of acceptability.

The overall acceptability calculated was made up of: texture 40% and taste, odour, colour and appearance each with 15%. The judges were also asked to comment on odour, texture, colour and flavour and the reason for rejecting a particular sample. The descriptors used for the sensory characteristics were developed during training sessions and previous taste panels in co-operation with the panellists.

## 2.5. Statistical analysis

The significant effects of temperature and storage time on the quality of cold-smoked salmon, as measured by the chemical, microbiological and sensory evaluations, were determined by the ANOVA method using STATISTICA program vs. 5.1 (1997) at  $p \leq 0.05$ .

# 3. Results and discussion

# 3.1. Chemical analyses

#### 3.1.1. Hypoxanthine (Hx)

Post mortem degradation of ATP in fish muscle occurs due to endogenous enzymatic activity. This degradation goes through the intermediate products ADP, AMP, IMP, INO and Hx (Church, 1998). Hypoxanthine accumulation in fish tissue reflects the initial phase of autolytic deterioration, as well as bacterial spoilage (Woyewoda, Shaw, Ice, & Burns, 1986).

Fig. 1 shows increasing levels of Hx from initial values of 0.29–1.17 µmol/g of salmon to final values of 1.03, 0.67, 2.24, 0.85 and 0.66 µmol/g of salmon after 26, 21, 20, 10 and 7 days at 0, 2, 4, 6 and 8 °C, respectively. Truelstrup et al. (1995) found higher levels of Hx ranging from an initial 1.6–3.0 to 5–7 and 7–8 µmol/g at 5 and 10 °C, respectively, in cold-smoked salmon. In this study, the amount of Hx increased significantly ( $p \le 0.05$ ) with greater storage time, increasing temperature and greater TVC, aerobic and anaerobic.

Bitter off-flavours were characteristic of the spoiling fish and have been connected with Hx production (Fletcher, Summers, & Van Veghel, 1998). This was also observed in our research study. Truelstrup et al. (1995), considered Hx as the best objective indicator for sensory quality of cold-smoked salmon.

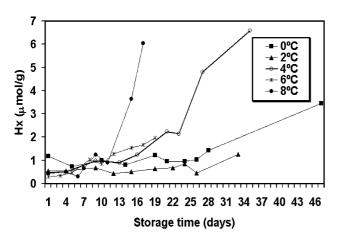


Fig. 1. Changes in hypoxanthine (Hx) in vacuum-packed cold-smoked salmon during refrigerated storage.

# 3.1.2. K value

Fig. 2 shows that initially K values were between 35.7% and 53.4% and, at the end of shelf life, these values were 72.6%, 69.6%, 97.8%, 63.2% and 69.8% after 26, 21, 20, 10 and 7 days at 0, 2, 4, 6 and 8 °C, respectively.

The *K* value, correlated closely with the storage time of fresh modified atmosphere-packed salmon at 2 °C (De la Hoz, López-Galvez, Fernández, Fierro, & Ordóñez, 2000) but highly variable values were observed in another study (Randell, Hattula, Skytt, Siverstvik, & Bersgslien, 1999).

Erikson, Beyer, and Sigholt (1997), proposed a K value of up to 70–80% in order to assure good quality in iced-stored fillets of Atlantic salmon. It was found that K value increased significantly with storage time and decreased significantly with sensory evaluation ( $p \leq 0.05$ ). This was also observed by Clark (1996) and Poblete (1997) in fresh salmon fillets under refrigerated storage.

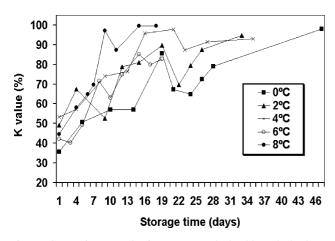


Fig. 2. Changes in % K value in vacuum-packed cold-smoked salmon during refrigerated storage.

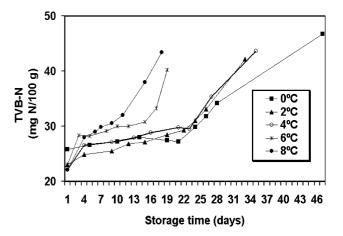


Fig. 3. Changes in total volatile bases (TVB) in vacuum-packed coldsmoked salmon during refrigerated storage.

# 3.1.3. Total volatile bases

TVB increased from initial values of 22.1–25.8 mg TVB-N/100 g to 31.8, 29.3, 29.8, 30.0 and 29.9 mg TVB-N/100 g at 0, 2, 4, 6 and 8 °C after 26, 21, 20, 10 and 7 days, respectively (Fig. 3). The limit established by the Chilean fishing authority (Sernapesca, 1996) is 30 mg TVB-N/100 g. Levels from  $15.5 \pm 0.3$  to  $52.8 \pm 2$  mg TVB/100 g, with a maximum production rate during the last 2 weeks, were found by Leroi, Joffraud, Chevalier, and Cardinal (1998), in cold-smoked salmon at 8 °C.

The increase in TVB was caused by a combination of microbiological and autolytic deamination of amino acids and the complete microbial reduction of TMAO to TMA (Truelstrup et al., 1996). According to Leroi et al. (1999), the higher producers of TVB in cold-smoked salmon were Enterobacteriaceae, *Photobacterium* spp. and *Lactobacillus* spp.

The influence of storage temperature was significant  $(p \le 0.05)$  for TVB. The TVC, aerobic and anaerobic, showed a positive correlation, at all temperatures, with TVB  $(p \le 0.05)$ . Sensory evaluation was also significant  $(p \le 0.05)$  for TVB at any storage temperature.

#### 3.1.4. Trimethylamine

Concentrations of TMA ranged from 2.9 to 3.5 mg TMA-N/100 g in fresh cold-smoked salmon to 10.2, 7.3, 7.5, 7.4 and 7.7 mg TMA-N/100 g at 0, 2, 4, 6 and 8 °C after 26, 21, 20, 10 and 7 days, respectively (Fig. 4). Similar results were found by Leroi et al. (1998), over 5 weeks of cold-smoked salmon vacuum storage at 8 °C, meaning that TMA did not play a major role in the spoilage mechanisms as only small amounts of TMA were produced.

The final concentration and the rate of TMA development depended upon storage temperature; increasing storage temperature resulted in higher TMA concentration.

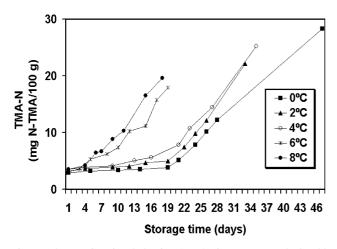


Fig. 4. Changes in trimethylamine (TMA) in vacuum-packed coldsmoked salmon during refrigerated storage.

All of these results, located at the shelf life borderline, are below the limit indicated by Sernapesca (1996) of 15 mg TMA-N/100 g. No legal limits for TMA-N have been set in the European Union (CEE, 1991). Changes in TMA correlated well with sensory scores, storage temperature, storage time and TVC aerobic and anaerobic ( $p \le 0.05$ ).

#### 3.1.5. Biogenic amines

Biogenic amines are usually associated with fish deterioration of the Scombridae family species (Hwang, Chang, Shian, & Cheng, 1995). The presence of biogenic amines is related to both microbial decarboxylation of their precursor amino acids and temperature abuse during post-catch handling (Veciana et al., 1995). Histamine intoxication, or scombroid fish poisoning, is the most common health problem related to high contents of biogenic amines in fish (Kim et al., 2002).

Initial values for putrescine were lower than 6 ppm and increased exponentially at the end of the different storage periods (Fig. 5). This behaviour was also observed by Mietz and Karmas (1977). Production of putrescine was enhanced 10–15 times when cultures of *Serratia liquefaciens* or *Hafnia alvei* were grown with *Carnobacterium divergens* or *Lactobacillus sakei* subsp. *carnosus* (Jorgensen, Huss, & Dalgaard, 2000).

Fig. 6 shows the concentration of cadaverine throughout the storage time at the range of 0-8 °C. Also, the levels increased markedly after the end of shelf life.

Cantoni, Moret, and Comi (1993) found low levels of cadaverine in smoked-salmon; however, in spoiled samples the measured levels of this amine were above 250 ppm. Lower levels were obtained in this research. However, at 8 °C, histamine levels (Fig. 7) ranged from below 6 to 22.1 ppm at the beginning of storage. Throughout the storage time, fluctuations were observed, except at 0 °C.

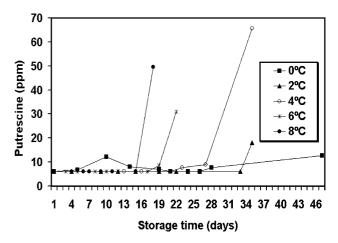


Fig. 5. Changes in putrescine in vacuum-packed cold-smoked salmon during refrigerated storage.

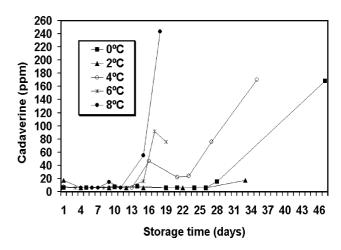


Fig. 6. Changes in cadaverine in vacuum-packed cold-smoked salmon during refrigerated storage.

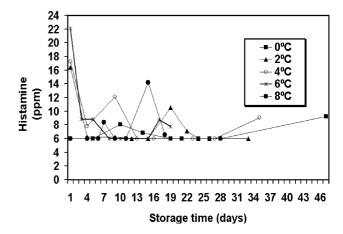


Fig. 7. Changes in histamine in vacuum-packed cold-smoked salmon during refrigerated storage.

The production of biogenic amines during chill storage (5 °C) of cold-smoked salmon from 3 smokehouses over a 2 years period (1997 and 1998) was studied by Jorgensen et al. (2000). Results of the study showed that

10

the production of biogenic amines is unlikely to result in histamine poisoning in humans, as indicated by epidemiological data. Some samples exceeded the defect action level of 50 ppm established by the FDA for Scombridae and 100–200 ppm by E.U. regulations for Scombridae and Clupeidae, but no samples reached toxic levels of 500 ppm, a value at which one would expect to encounter illness and that the FDA would use in legal proceedings (EEC, 1991; FDA, 1998). Sernapesca (1996) also established limits for the histamine level in fish to be between 100 and 200 ppm.

Mackerel, albacore and mahi–mahi have shown to be good substrates for histidine decarboxylation by Morganii at an elevated temperature (15 °C); salmon was the fish species least susceptible to histamine formation, probably due to low content of free histidine in the muscle (Kim et al., 2002). *Photobacterium phosphoreum* was the only species that produced histamine when inoculated on sterile cold-smoked salmon (Jorgensen, Huss, & Dalgaard, 2000).

Polyamines are useful for judging the hygienic quality but are not good indicators of early freshness of fish (Mazorra, Pacheco, Diaz, & Lugo, 2000).

# 3.2. Microbiological analyses

10 9

8

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6

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0

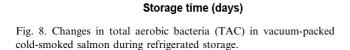
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Log TAC (CFU/g)

Results of total aerobic (TAC) and anaerobic (TANC) bacterial plate counts made on cold-smoked salmon are shown in Figs. 8 and 9.

Total aerobic count at the beginning of storage ranged from 150 to  $174 \times 10^3$  CFU/g. At the end of shelf life, after 26, 21, 20, 10 and 7 days at 0, 2, 4, 6 and 8 °C, respectively, levels of  $185 \times 10^4$ ,  $303 \times 10^5$ ,  $450 \times 10^4$ ,  $<300 \times 10^6$  and  $760 \times 10^3$  CFU/g were reached.

However, Sernapesca (1996), established limits for TVC between  $10^5$  and  $5 \times 10^5$ . Truelstrup et al. (1995) also found that cold-smoked salmon with a high number of microorganisms ( $10^8$  CFU/g) was not always spoiled.



7 10 13 16 19 22 25 28 31 34 37 40 43 46

0°C

2°C

4°C

-6°C

8%

9 8 Log TANC (CFU/g) 7 6 5 - 0°C 2°C 4 4°C 3 6°C 2 + 8°C 1 0 7 10 13 16 19 22 25 28 31 34 37 40 43 46 1 - 4 Storage time (days)

Fig. 9. Changes in total anaerobic bacteria (TANC) in vacuum-packed cold-smoked salmon during refrigerated storage.

TANC ranged from 50 to  $194 \times 10^3$  CFU/g at the beginning of storage and after 26, 21, 20, 10 and 7 days at 0,2, 4, 6 and 8 °C, levels of  $196 \times 10^4$ ,  $220 \times 10^4$ ,  $380 \times 10^4$ ,  $<300 \times 10^3$  and  $590 \times 10^3$  CFU/g were found, respectively.

There was a positive correlation for both TAC and TANC, with storage temperatures and time ( $p \le 0.05$ ). However a significant reduction in mean sensory scores ( $p \le 0.05$ ) was observed.

The initial total coliform count (TCC) was <3 MPN/g (Fig. 10). TCC increased after shelf life storage up to >4 to 43 NMP/g salmon. The limits established by Sernapesca (1996) are 4 MPN/g and 10 MPN of *Escherichia coli*, respectively. No significant correlation ( $p \le 0.05$ ) was found between TCC and storage time and temperature.

Staphylococcus aureus, L. monocytogenes, C. botulinum and Salmonella were not detected in cold-smoked salmon throughout storage time at 0–8 °C.

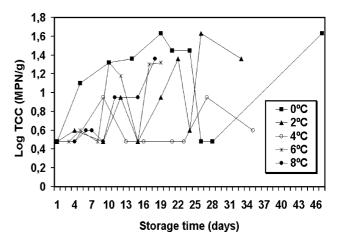


Fig. 10. Changes in total coliform bacteria (TCC) in vacuum-packed cold-smoked salmon during refrigerated storage.

Yeast and mold levels were low for cold-smoked salmon. Truelstrup et al. (1996) have reported that yeasts and molds were not considered to be of any importance for spoilage of this kind of product.

Lactic acid bacteria dominated the microflora throughout the storage period (Fig. 11). This was also reported by Leroi, Arbey, Joffraud, and Chevalier (1996, 1998). The lactic acid bacteria group (LAB) is known to produce organic acids and ethanol as typical fermentation end products (Gottschalk, 1986). There are no reported observations on the lactic acid bacterial ability to produce Hx or TMA. In the case of smoked-salmon, the roles of the microflora are not as clear as many authors have shown, in that there is no correlation between shelf life and LAB count, or any other bacterial number (Dodds, Brodsky, & Warburton, 1992). They may be found in high numbers before the product is spoiled (Truelstrup et al., 1996).

Lactobacillus spp., showed significant correlation  $(p \leq 0.05)$  with storage time and sensory quality at all storage temperatures.

Differences among the microflora isolated from three different smokehouses and production batches, indicate that the existence of one typical microflora including one specific spoilage organism for cold smoked-salmon is unlikely. The microflora at the time of spoilage are highly variable with lactic acid bacteria, Enterobacteriaceae and marine vibrios among the dominating microorganisms (Truelstrup, Drewes, & Huss, 1998).

The bacteria mainly responsible for spoilage in sterile cold-smoked salmon stored in vacuum packs at 6 °C were *L. sakei*, *L. farciminis* and *B. thermosphacta*, which produced sulphurous, acidic and rancid off-odours, respectively. Some strains of *S. liquefaciens* produced rubbery, cheesy or acidic off-flavours. Some *P. phosphoreum* isolates were characterized by an acidic effect (Stohr, Joffraud, Cardinal, & Leroi, 2001).

In cold-smoked fish, aerobic conditions lead to faster spoilage than under vacuum or MAP packaging. Under aerobic conditions, *Pseudomonas*, yeasts and some lactic acid bacteria developed, whereas anoxic packaging conditions resulted in development of lactic acid bacteria flora with a minor component of Gram-negative bacteria. Typically, shelf life is reduced by a factor of 1.5 to 2 by aerobic storage as compared to vacuum-packed storage. (Dufresne et al., 2000; Gram, 2001).

## 3.3. Sensory evaluation

Results of the sensory analyses of cold-smoked salmon at 0, 2, 4, 6 and 8 °C are shown in Fig. 12. With increasing storage temperatures, decreased shelf life was obtained. The spoilage patterns described by the panellists were: softening of texture before off-odours developed and presence of bitter, sour, rancid and ammonia off-flavours. Appearance score decreased as colour discolouration increased. Autolytic enzymes can have a major impact on the loss of textural quality of cold-smoked salmon during the early stages of deterioration, but they did not produce the characteristic off-flavours and off-odours, which are typical of microbiological activity (Truelstrup et al., 1996). Shelf life values were 26, 21, 20, 10 and 7 days for samples stored at 0, 2, 4, 6 and 8 °C, respectively.

Truelstrup et al. (1998), found that the estimated shelf life at 5 °C of vacuum-packed slices of cold-smoked salmon ranged between 21 and 36 days and for whole fillets between 32 and 49 days. Shelf lives of whole fillets were always longer than shelf lives of the corresponding sliced products. It may, however, be difficult to compare salmon produced by different smokehouses because a number of important factors, such as packaging material, production method, size and composition of initial

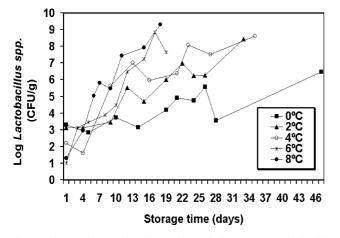


Fig. 11. Changes in *Lactobacillus* spp. bacteria in vacuum-packed coldsmoked salmon during refrigerated storage.

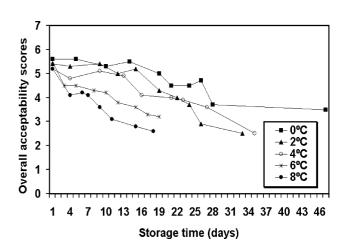


Fig. 12. Changes in total sensory scores in vacuum-packed coldsmoked salmon during refrigerated storage.

microflora, numbers of freezing and thawing cycles and quality of raw material, varied and this may explain why salmon with 4.1% and 6.1% salt in the water phase, had comparable shelf lives.

For French vacuum-packed cold-smoked salmon stored at 5 °C, the shelf life was very variable (1–6 weeks) and was related to the initial Enterobacteriaceae load, depending on hygienic conditions in the smokehouse rather than on the material quality or the processing parameters (Leroi, Joffraud, Chevalier, & Cardinal, 2001). Estimation of cold-smoked salmon quality is possible by measuring three parameters: lactobacilli and yeast counts and TVB concentration.

Significant correlations ( $p \le 0.05$ ) were found between storage time, sensory quality and TVB, TMA, K value, *Lactobacillus* spp., TAC and TANC at different storage temperatures. These indicators were found to be superior as an indicator of quality because a clear relation to sensory evaluation could be established.

## 4. Conclusions

According to sensory analysis, vacuum-packed coldsmoked salmon presented a shelf life of 26 days at 0 °C, 21 days at 2 °C, 20 days at 4 °C, 10 days at 6 °C and 7 days at 8 °C.

TVB, K value, TAC, TANC and Lactobacillus spp., were the most suitable indicators to determine coldsmoked salmon freshness. There was a significant correlation of these variables (p < 0.05) with storage time and sensory analysis at different storage temperatures.

Hypoxanthine, TCC, molds, yeasts and biogenic amines were not useful deterioration indicators of cold-smoked salmon at temperatures in the range of 0–8 °C.

Pathogenic microorganisms such as *L. monocytogenes*, *C. botulinum*, *Salmonella*, Coliform and *S. aureus* were not detected in cold-smoked salmon.

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