



A comprehensive approach to assessment of the quality and shelf-life for retail fresh hake (*Merluccius australis*) during Chilled Storage

Carmen Lopez-Joven^{a,*}, Reydoret Umaña^{a,b}, Paola Guajardo^a, Ignacio de Blas^c

^a Instituto de Medicina Preventiva Veterinaria, Facultad de Ciencias Veterinarias, Universidad Austral de Chile, Saelzer Building 5° Floor, Campus Isla Teja, 5090000 Valdivia, Chile

^b Laboratorio de Alimentos y Aguas, Facultad de Ciencias Veterinarias, Universidad Austral de Chile, Saelzer Building 5° Floor, Campus Isla Teja, 5090000 Valdivia, Chile

^c Department of Animal Pathology, Faculty of Veterinary Sciences, Instituto Universitario de Investigación Mixto Agroalimentario de Aragón (IA2), Universidad de Zaragoza, Zaragoza, Spain

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ABSTRACT

The Chilean southern hake, *Merluccius australis*, is one of the most important marine fishery resources in Chilean Patagonia. The aim of this study was to investigate the compositional changes in *M. australis* due to alterations in its volatile composition and other parameters over a period of 23 days in order to evaluate its shelf-life storage at chilled temperatures using fish quality parameters. The samples were bought from the main seafood market in Valdivia city, located in southern Chile.

First, an organoleptic evaluation of sensory properties was conducted. Then, pH, total volatile basic nitrogen contents (TVB-N), and trimethylamine (TMA) levels, histamine concentration, and total viable counts (TVC) levels as a microbiological parameter were determined. A repeated measures generalized linear model (GLM) and Duncan's test as post hoc test were used to analyze data.

The data revealed that pH levels were always over 7 from day 0 (7.31) to 23 (7.72), which could indicate the prevalence of good but not highest quality in specimens at day 0. No significant differences ($P = 0.558$) in pH values were obtained during all days of storage at lower than 4 °C.

Additionally, the study found a progression of volatile bases levels where TVB-N and TMA levels, increased significantly after 7 days of storage in both cases and exceeded the allowable limit to the family *Merlucciidae* at day 15 and at day 9, respectively. Both could be considered as reliable indicators by freshness in Chilean southern hake under the present scenario, showing the first empirical results at long storage. On the other hand, histamine concentration was below the allowable limit in fish. And total viable counts (TVC) levels as a microbiological parameter, fluctuated and increased gradually during the first 9 days (from 4.18 log cfu/ml to 4.91 log cfu/ml), and increasing significantly after 15 days (5.42 log cfu/ml) and 23 days (6.32 log cfu/ml) of storage, but did not exceed the spoilage regulation thresholds. These findings emphasize the importance of implementing good practices and improving knowledge in the retail market and to consumers to avoid lengthy storage of fresh fish before consumption, to prevent foodborne illnesses.

1. Introduction

Fish freshness is one of the most important attributes to define the quality of fish products and its market value. Many factors during *postmortem* storage contribute to the deterioration of fish quality, starting immediately after fishing due to the occurrence of a series of autolytic processes that lead first to *rigor mortis*, and afterwards, to autolysis of proteins and fats. This creates a favorable environment for

bacteria and the action of their autolysis enzymes as well as hydrolytic enzymes on the fish muscle (García et al., 2015; Venugopal, 2002). In this context, environmental and feeding habits of different fish species are relevant, so it is important to provide a reliable freshness indicator to predict fish quality and safety, considering the complexity of quality deterioration in the different fish flesh species (Pérez-Villarreal & Pozo, 1990; Jouki et al., 2014).

Fish freshness can be evaluated using several methods; some of them

* Corresponding author.

E-mail address: carmen.lopez@uach.cl (C. Lopez-Joven).

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should be determined together. In this context, a chemical change method used as an index for freshness of fish, is focused on the total volatile basic nitrogen (TVB-N) content of the fish (Stansby et al., 1944) which included the total amount of ammonia (NH₃), dimethylamine (DMA) and trimethylamine (TMA). Also, changes in TMA content and pH value can be evaluated as a chemical method to indicate fish spoilage. These chemical changes in stored fish products lead to short shelf life and economic losses (Horsfall et al., 2006). On the other hand, to assess fish safety, regulations also establish the evaluation of histamine, one of the most important and toxic biogenic amines which are formed in food products during storage and may cause food poisoning in humans when contained at a high level. It is produced during bacterial decarboxylation of the histidine present in fish muscles (Davidek & Davidek, 1995).

Chemical methods can be complemented with microbiological parameters as total viable counts (TVC) related to spoilage bacterial loads, and the organoleptical evaluation focused on key sensory attributes such as general appearance as skin pigmentation and mucus; eyes; gills; consistency like firmness and elasticity of muscle; and abdominal cavity (cleanliness and presence of residual organs) at different storage intervals for evaluating the freshness of chilled hake along storage. However, the last one requires a trained panel to evaluate all these organoleptical attributes and it not always available at different steps of the fishery chain.

In Chile, Chilean southern hake (*Merluccius australis*) is one of the most important marine fishery resources in Chilean Patagonia; its production come from artisanal fisheries (4525 ton in 2021) in Los Lagos Region and industrial trawlers in Aysén Region (4897 ton in 2021) (SERNAPESCA, 2021). Each year, two third of the landed product are demanded in markets such as Spain, Poland, Portugal, Brazil, and the United States, and they must be within the limits established for the different parameters inside the legislation of the final country (SERNAPESCA, 2023).

The risk in products placed on the retail market based on their good or bad hygienic practices, is not currently assessed, and this emphasizes the need for a better knowledge of freshness parameters in seafood products (Taliadourou et al., 2003; Ózogul et al., 2006).

At present more reliable indicators by freshness in Chilean southern hake at long storage are needed and are considered here. Therefore, the aim of the present study was to investigate the compositional changes occurring in this Chilean southern hake during a period of 23 days, with the determination of the maximum acceptable TVB-N levels and other freshness parameters as TMA-N, pH, histamine and TVC limits and organoleptical attributes in order to evaluate its shelf-life storage and also, to determine if they will be very reliable indicators by freshness in Chilean southern hake.

2. Materials and methods

2.1. Fish samples collection and storage conditions

Fresh gutted medium-sized Chilean southern hake (average weight and length: 1500 g and 70 cm) caught in Los Lagos waters were purchased from the retail market in Valdivia (Los Rios Region, Chile). Hakes were brought to the laboratory within 30 min in isothermal containers. Once in the lab, two specimens were analyzed to assess initial quality. Other hakes were packed and stored into insulated polystyrene boxes under refrigeration conditions in cold chamber with temperature lower than 4 °C (fish temperature ranged between 0.8 to 3.9 °C) for subsequent organoleptically, chemical and microbiological analyses for 23 days. This temperature is consistent with the refrigeration temperature of consumers in their homes.

These boxes are similar to those in use during the process of marketing fish. Two fish specimens were taken out of refrigerated storage and were organoleptical, chemical, and microbiological analyzed at days 1, 2, 3, 4, 5, 6, 7, 8, 9, 15, and 23. Chemical analyses were

performed twice, since microbiological analyses were performed in quadruplicate.

2.2. Organoleptical evaluation

At each storage time, fish freshness was organoleptically assessed by panelists from the staff of Food and Water Laboratory, Austral University of Chile, according to National Chilean Regulation (MINSAL, 2019) and using the freshness ratings for fish set out in the Safety and Certification Manual for fresh and refrigerated fish for human consumption (SERNAPESCA, 2023). The Manual establishes three categories: highest quality (Extra), good quality (A), and fair quality (B). Unacceptable quality (C) was included according to guidelines presented in EC (1976) and EC (1989) (Table 1). Organoleptical assessment of the fish included the following parameters: general appearance (surface appearance as skin pigmentation and mucus), eyes, gills, consistency (firmness and elasticity of muscle), and abdominal cavity (cleanliness and presence of residual organs).

2.3. pH determination

The determination of pH along the storage time was carried out using the destructive technique according to Chilean Standard 2738. Official 2002 (INN, 2002a). The evolution of pH values of each fish was determined using a pH meter (ORION Benchtop 420A pH/mV Meter; Thermo Scientific, IL 60,061 United States) which was calibrated in buffers at pH 4.0 and 7.0 at room temperature. Briefly, a representative portion of the fish muscle (at least 200 g) was taken and homogenized for 1 min using a blender. Then, a 100 ml boiled and cooled to 20 °C distilled water was added to 5 g of the previously homogenized sample and situated in an Erlenmeyer, and shaken for 30 min. Finally, collected supernatant was obtained and the pH measurement was performed. Duplicate readings were obtained from sample and then averaged.

2.4. Determination of total volatile basic nitrogen (TVB-N)

The TVB-N content was determined using a steam distillation method according to Chilean Standard 2668. Official 2018 (INN, 2018).

Briefly, 25.5 ± 0.5 g of fish were blended with 80 ml of trichloroacetic acid (TCA) 5 %. The supernatant was placed in a dry centrifuge tube and centrifuged for 5 min at a speed of 2500 rpm. Subsequently, 20 ml of the centrifuged liquid was pipetted to reach a full volume of approximately 100 ml, with water for analysis and drops of antifoam added. Then, 2 g of magnesium oxide was added and immediately passed to the distilled phase. The distillate was collected in an Erlenmeyer in which there was 30 ml of boric acid solution and sufficient water for analysis having as precaution that the stem of the refrigerant was not <10 mm in the boric solution. The distillation was stopped once at least 150 ml of distillate had been collected. Finally, the titration was carried out by adding standardized 0.1 N sulfuric acid solution and the volume used in the titration (V) was recorded in ml. Each analysis was repeated twice. The results were expressed as mg TVB-N/100 g muscle.

2.5. Determination of trimethylamine nitrogen (TMA-N)

Trimethylamine nitrogen (TMA-N) values were determined according to Chilean Standard 2757. Official 2002 (INN, 2002b). This technique involves the preparation of a 7.5 % trichloroacetic acid solution (TCA) and 0.02 % picric acid. Briefly, 25 g of fresh fish sample were weighed into the blender joined with 50 ml of a 7.5 % TCA solution; the mixture was shaken and filtered. Then, 4 ml of this filtrate was used to react with the organic solvent toluene which extracts the analyte in basic medium. From the organic phase 5 ml was used to react with picric acid. A calibration curve using trimethylamine 0.01 mg/ml as reference material was used to the quantification. Data were expressed as mg TMA/100 g muscle.

Table 1

Scale employed for evaluating the freshness of chilled hake along storage (European Commission [EC], 1976; SERNAPESCA, 2023).

Attribute	Highest quality {Extra}	Good quality {A}	Fair quality {B}	Unacceptable {C}
General appearance (surface appearance)	Very intense pigmentation; transparent mucus; medallions and fillets: rosy translucent colour; absence of parasites visible.	Insignificant pigmentation losses; significant pigmentation losses by mechanical factors; milky mucus; absence of visible parasites.	Minor injuries; pigmentation without shine; slightly opaque-greyish mucus; absence of visible parasites.	Widely opaque mucus; important pigmentation losses; sour and putrid external odour.
Eyes	Convex; transparent cornea; bright and black pupil.	Convex and slightly sunken; slightly opalescent cornea; black and cloudy pupil.	Flat; opalescent cornea; opaque pupil.	Concave and milky cornea; Internal tissues blurred.
Gills	Brightly red; without odour; lamina perfectly separated.	Pink coloured; without odour; lamina adhered in groups.	Slightly pale; incipient fishy odour; lamina adhered in groups.	Grey-yellowish colour; intense ammonia odour; lamina totally adhered.
Consistency	Presence or partial disappearance of rigor mortis symptoms.	Firm and elastic; pressure signs disappear immediately and completely.	Presence of mechanical signs; elasticity notably reduced.	Important shape changes due to mechanical factors.
Abdominal cavity	Stains of red blood bright; serous clear; absence of visible parasites.	Stains of red blood; serous clear; absence of visible parasites.	Traces of brownish-red blood; cloudy serosa; absence of visible parasites.	Residues of organs and should be brownish in colour. Opaque flesh or in a more advanced state of decay.

2.6. Determination of histamine

The histamine content was determined according to Chilean Standard 2637. Official 2001 (INN, 2001), with minor modifications. The methodology used High-Performance Liquid Chromatography with UV detection and DAD detector (HPLC-DAD). This methodology possesses high sensitivity and accuracy, and it was based on extraction with trichloroacetic acid and subsequent quantification by derivatization using dansyl reaction. Finally, chromatographic analysis was performed and the mobile phase used was acetonitrile, water and methanol, using a proportion (%) 50/25/25, respectively. Standard and calibration curve were prepared. Histamine was detected at the wavelength of 254 nm.

2.7. Microbiological analyses

The measurement of bacterial counts was conducted according to Chilean Standard 2659. Official 2002 (INN, 2002c). Briefly, 10 g of each fresh fish sample were removed aseptically to sterile bags containing 90 ml of phosphate buffer, and then were homogenized in a stomacher (ESH, Merck, Germany) for 1 min. Thereafter, serial decimal dilutions were undertaken using phosphate buffer to determine total viable counts (TVC). Each sample was seeded in duplicate by depositing 1 ml in sterile Petri dishes and then pouring 15 ml of plate count agar (Merck, Germany) maintained at 46 ± 1 °C in the thermoregulated bath. It was mixed and distributed homogeneously, and the plates were allowed to solidify. They were incubated at 35 ± 1 °C for 48 ± 2 h.

Then colonies were counted by selecting plates with no invasive growth which contained between 25 and 250 colonies. The results were expressed as cfu/g of sample. Each analysis was repeated twice, and two technical replicates were obtained for each one.

2.8. Statistical analysis

A repeated measures generalized linear model (GLM) was used to analyze data from the different measurements, chemical and microbiological results as dependent variables, and time as the independent variable. Duncan test was selected as *post-hoc* test. The IBM SPSS 26.0 for Windows Software (SPSS Inc., Chicago, IL, USA) was used to carry out the statistical analysis. The alpha error was set up at 0.05 in all cases.

3. Results and discussion

Fish is one of the most highly perishable food products and is highly susceptible to deterioration immediately after harvest, a consequence of series of complicated deteriorative changes brought about by its own enzyme, by bacteria and by chemical action (Shewan, 1976; Ali et al.,

2010). Also, lack of adequate fish handling, processing techniques and storage facilities contribute significantly to the increase of its contamination levels and subsequent decomposition. Our results may not reflect the maximum shelf-life potential of freshly caught *Merluccius australis* from sea to laboratory, but they show the real situation of retail market in southern Chile. These results could be compared to other retail markets which have the same dynamics to inadequate practices.

3.1. Organoleptical evaluation

Results of the organoleptical evaluation are shown in Table 2. Hake fish maintained good quality (E and A categories) up to day 1. At days 2 and 3, all factors still obtained an "A" score, except eyes factor which obtained a "B" score, acquiring a change in shape (flattened) and color of the cornea. At day 4, almost all factors obtained an "B" score. After this time, quality decreased, and at day 5 of refrigeration, the eyes factor obtained a "C" score (rejection) acquiring a concave shape and opaque cornea. Also, changes in the external appearance of the specimens were observed, mainly in the brightness of the skin, mucus, and in the ease with which the scales were removed. In addition, they present a soft consistency when the musculature is pressed with the finger, the impression is marked for a few seconds and then returns to normal. The gills also begin to take on a paler color, adhering to each other and irregularly shaped.

At day 7, 8 and 9 of refrigeration, both sensory parameters, eyes and gills, were limiting factors and obtained a "C" score (rejection). Gills acquired dull and pale brown coloration and started to present mucus, slight bad odor, and the lamellae were completely adhered to each other in a totally irregular way. The other three factors, general appearance,

Table 2

Organoleptical evaluation of hakes during its chilled storage. Freshness categories are as expressed in Table 1. Letters correspond with Highest quality (E), Good quality (A), Fair quality (B), Unacceptable quality (C).

Storage time (days)	General appearance	Eyes	Gills	Consistency	Abdominal cavity
0	A	A	A	E	E
1	A	A	A	E	A
2	A	B	A	A	A
3	A	B	A	A	A
4	B	B	B	B	A
5	B	C	B	B	B
6	B	C	B	B	B
7	B	C	C	B	B
8	B	C	C	B	B
9	B	C	C	B	B
15	C	C	C	C	C
23	C	C	C	C	C

consistency, and abdominal cavity still obtained a “B” score.

After 15 days of refrigeration the specimens were not suitable for consumption. All organoleptical parameters were limiting factors and obtained a “C” score (rejection). Abundant yellow mucus was observed on the surface, leading to a disagreeable odor, scales that came off very easily to the touch, the consistency of the musculature was extremely soft, presenting a marked fingerprint for a much longer period that finally did not return to normal. The eyes were totally sunken and dehydrated, with complete corneal opacity, and the gills were brown, dull, with a lot of mucus, an extremely unpleasant odor and lamellae completely adhered to each other. Finally, after 23 days of refrigeration, the sample evaluated was completely putrefied.

Previous research that used ice storage (as slurry and/or flakes ice) showed significantly higher shelf-life times for medium-sized fish species, such as albacore, hake, and turbot. In this context, Ruiz-Capillas and Moral (2001) indicated that hake gutted specimens (average weight: 420 g) could be fit for human consumption until day 19 of storage in ice. Rodríguez et al. (2003) showed the turbot specimens not headed or gutted (average weight: 1750 g) stored in ice was being rejected on day 22 because of segregation of mucus from the skin, leading to a disagreeable odor. One year later, Rodríguez et al. (2004) showed the good sensory quality, categories E and A, of hake specimens stored in flake ice was only maintained up to day 2, and hake specimens being rejected on day 8, while the good sensory quality of hake specimens stored in slurry ice was maintained up to day 12. On day 15 and onwards the hake specimens were no longer acceptable.

3.2. Evolution of pH values to define the quality of fresh fish

The present study shows that pH values for hake stored under chilled temperatures increased progressively throughout the experiment from the initial 7.31 to values of 7.36 and 7.53 after 4 and 7 days of storage, respectively. The pH values reached 7.77 and 7.72 after 15 and 23 days, respectively (Fig. 1). However, no significant differences ($P = 0.558$) in pH values were obtained during all days of storage at lower than 4 °C.

This could suggest that the *postmortem* glycolysis phenomenon has occurred prior to commercialization, considering that the pH immediately after rigor mortis has resolved is usually between 6.2 to 6.5 (depending upon fish species) (Pedrosa-Menabrito & Regenstein, 1988). Adequate ultimate pH for the meat of unstressed hake specimens ranges

around 7.0, so pH values higher than 7 may limit its consumption (Moral, 1987; Ruiz-Capillas & Moral, 2001).

Here, initial pH values were always over 7, which could indicate the fish have not been at its highest freshness state at the time of sampling. This could be attributed to partial degradation prior to acquisition at the retail market, and likely due to inadequate practices such as delays in cold chain management; which could speed up the accumulation of alkaline compounds, such as ammonia, mercaptan and TMA compounds, mainly derived from microbial action or enzymatic phenomena that increase pH by neutralizing the lactic acid previously formed (Hebardet et al., 1982). Rodríguez et al. (2004) showed that hake specimens stored in flake ice chilled temperatures increased pH values at 6.98 and 7.71 after 5 and 19 days of storage, respectively. So, it is very important to follow good practice.

3.3. TVB-N in *M. australis* stored under chilled temperatures

The TVB-N is considered a direct quality indicator of meat freshness/deterioration. In the present study, interaction between storage time and TVB-N values ($P < 0.001$; Fig. 2) was observed. The initial TVB-N values was 6.63 mg/100 g, indicative of good quality of the raw meat used in this study and it is lower to those found by other authors that worked with whole European hake (*merluccius* L.), such as Ruiz-Capillas and Moral (2001) who found 10.92 mg/100 g, Perez-Villarreal and Howgate (1987) who found 15 mg/100 g (these authors used hake fillets and not whole hake), or Sotelo et al. (1995) who found 18.50 mg/100 g.

The value for this indicator increased progressively throughout storage and was significant on day 7 of storage, in comparison to the first 4 days. Until day 9 the values were below the allowable limit of TVB-N in fish belonging to the family *Merlucciidae* and others, to 30 mg/100 g based on SERNAPESCA (2023) and 35 mg/100 g based on Directive 2074/2005/EC (EC, 2005b) and Directive 1022/2008/EC (EC, 2008) (Fig. 2). Values exceeded when storage time was prolonged on days 15 (86.24 mg/100 g) and 23 (1118.52 mg/100 g) of storage in this study, which is consistent with the reports of other authors (Ruiz-Capillas & Moral, 2001; Khoshnoudi-Nia & Moosavi-Nasab, 2019).

Present TVB-N value was higher than those detected by Ruiz-Capillas and Moral (2001) whose level after 25 days of storage was 44.45 mg/100 g in gutted hakes (*M. merluccius*). Similar results were detected by Rodríguez et al. (2004) whose level after 19 days of storage was 47

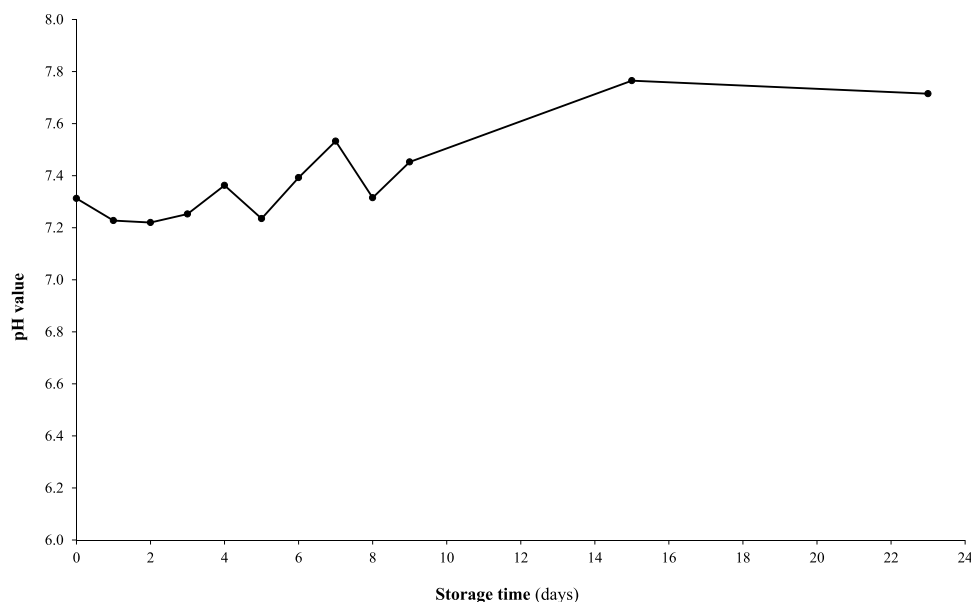


Fig. 1. Time evolution of pH content in *Merluccius australis* during 23 days storage at 4 ± 2 °C. Values are mean of duplicate determinations (with 2 replicates each one).

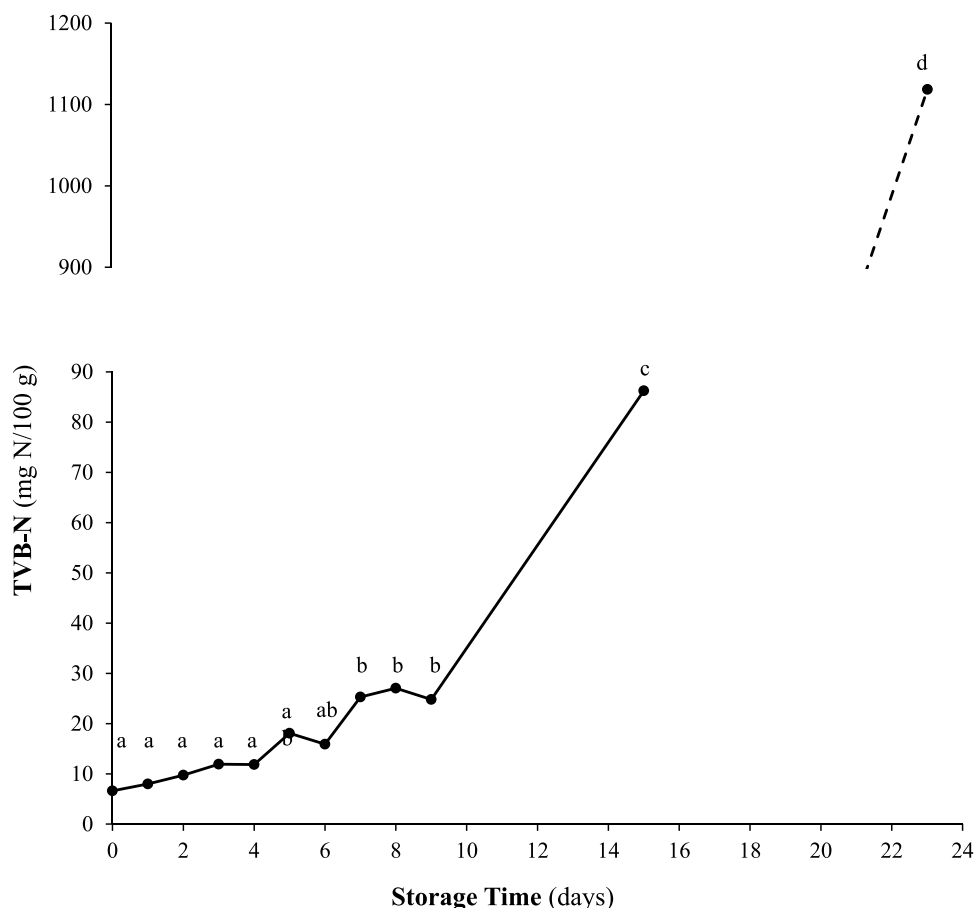


Fig. 2. Time evolution of TVB-N content in *Merluccius australis* during 23 days storage at 4 ± 2 °C. Different letters indicate significant differences ($P < 0.05$) according to Duncan *post-hoc* test. Values are mean of duplicate determinations (with 2 replicates each one).

mg/100 g in not gutted hakes (*M. merluccius*). In both studies fish specimens were placed in boxes with traditional flake ice in a refrigerated room at 2 °C, which could explain the lower results. Baixas-Nogueras et al. (2003) found lower TVB-N values from fresh and gutted hake (*M. merluccius*) stored in flaked ice and inside a refrigerator set at 4 °C, at day 14, where levels were almost 30 mg/100 g, which is below to the limit of acceptability established by the (European Commission EC, 2005b, 2008).

Besides hake, there are also other studies carried out with different fish species. A study realized by Li et al. (2023) focusing on large yellow croaker (*Larimichthys crocea*) stored during 4 °C refrigeration, also revealed a consistent increase in TVB-N, that exceeded the upper limit for acceptable quality (≤ 30 mg/100 g, according to the standard of SC/T 3101–2010; China Fisheries Industry Standard), on day 15, reaching 42.56 mg/100 g. On the other hand, a study carried out by Zheng et al. (2020) focused on Spanish mackerel (*Scomberomorus niphonius*) found an increasing trend in TVB-N during storage with a final value of 31.01 ± 0.58 mg/100 g on day 9, which exceeds the upper limit (25 mg/100 g).

3.4. Trimethylamine Nitrogen (TMA-N) in *M. australis* stored under chilled temperatures

Trimethylamine Nitrogen (TMA-N) is one of the best-known compounds produced during fish spoilage and it is mainly derived from bacterial breakdown of trimethylamine oxide (TMAO) which is an osmolyte naturally found in marine fish (Malle et al., 1986; Pedraso-Menabrito and Regenstein, 1990).

The results of analysis of TMA-N in fresh fish samples are shown in

Fig. 3. There was an interaction of storage time for TMA-N ($P < 0.001$). The initial TMA-N values were 0.45 mg/100 g, indicative of good meat quality (Pedrosa-Menabrito & Regenstein, 1990). The low production of volatile N compounds from day 0 to 4 would indicate the very limited growth of TMA-producing bacteria, such as *Shewanella putrefaciens*, *Photobacterium phosphoreum*, or certain species belonging to the families *Vibrionaceae* and *Enterobacteriaceae* (Rodríguez et al., 2003).

From day 7 to 9 the levels increase significantly, exceeding the maximum limit of TMA-N since day 9 (17.15 mg/100 g) based on SERNAPESCA (15 mg/100 g) (SERNAPESCA, 2023) or since day 7 (15.02 mg/100 g) based on Directive 91/493/EC (12 mg/100 g) (EC, 1991). On days 15 and 23 values increased significantly registered 82.28 mg/100 g and 652.46 mg/100 g, respectively.

Other reports concerning hake specimens (Ruiz-Capillas & Moral, 2001; Baixas-Nogueras et al., 2003) have described sharp increases in TMA-N contents after 12–14 days of storage in conventional flake ice; but always inside to the legal limit of acceptability established by SERNAPESCA (2023) and by the European Commission (EC, 1991). Similar results were obtained by Rodríguez et al. (2004) that found lower TMA-N values from not gutted hakes (*M. merluccius*) stored in flaked ice and inside a refrigerator set at 2 °C, with a final value of 11.5 mg TMA-N/100 g, after 19 days of storage.

The present study agrees with results obtained by Horsfall Jnr et al. (2023) on hake (*Merluccius capensis*) stored at -14 °C related to TVB-N and TMA-N parameters to evaluate fish spoilage indicators; although, the content of TMA-N ($3.25 [\pm 0.068]$ mg N/100 g) showed lower levels at 2-week considering that fishes were preserved at -14 °C over a period of seven weeks. In any case, the higher increase in TMA-N content is directly proportional to the increase in storage time and stored

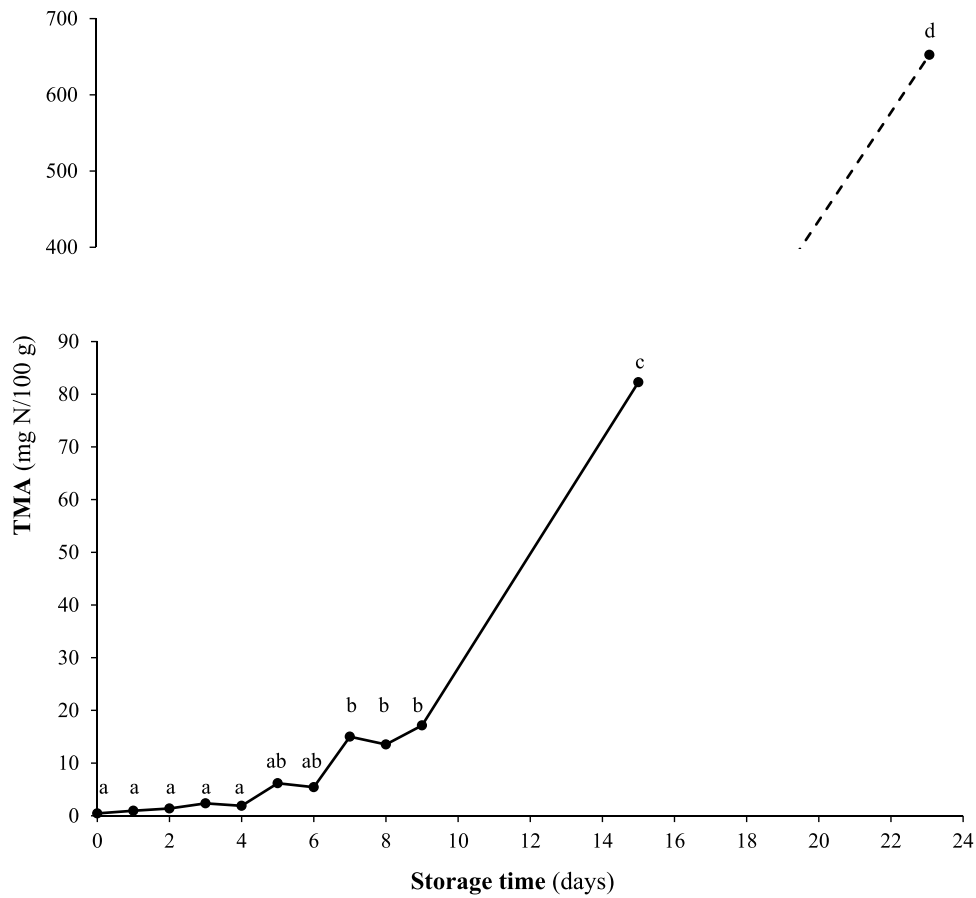


Fig. 3. Time evolution of TMA content in *Merluccius australis* during 23 days storage at $4 \pm 2^\circ\text{C}$. Different letters indicate significant differences ($P < 0.05$) according to Duncan *post-hoc* test. Values are mean of duplicate determinations (with 2 replicates each one).

temperature, which is a significant warning sign to consumers to resist lengthy storage before consumption.

3.5. Histamine in *M. australis* stored under chilled temperatures

Determination of histamine is important not only because of its toxicity for humans, but also as an indicator of the freshness of fish and

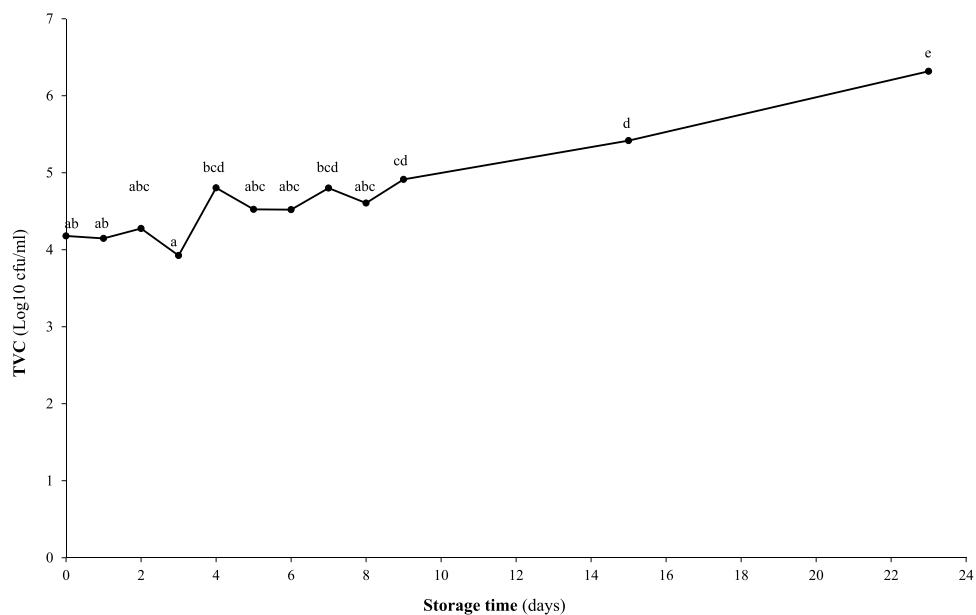


Fig. 4. Time evolution of TVC content in *Merluccius australis* during 23 days storage at $4 \pm 2^\circ\text{C}$. Different letters indicate significant differences ($P < 0.05$) according to Duncan *post-hoc* test. Values are mean of quadruplicate determinations (two determination for fish, with 2 replicates each one).

fish products (Karovičová & Kohajdová, 2005; Biji et al., 2016). Adequate refrigeration practices (respecting the time and low temperature) during unloading, transportation, storage, and processing of fish, are fundamental actions to prevent bacterial spoilage and production of histamine (Hungerford, 2010; McLauchlin et al., 2005). In the present study, histamine was detected in one sample (4.5 %) at time 15 days, and its concentration determined by HPLC-DAD was 19.8 mg/kg, which is below the allowable limit of histamine in fish and fish products to 100–200 mg/kg (European Commission EC, 2005a; SERNAPESCA, 2023).

3.6. Bacterial enumeration in *M. australis* stored under chilled temperatures

Bacterial loads of freshly harvested aquatic products are usually 3.00–4.00 log cfu/g; hence, the TVC content below 4.00 log CFU/g is generally regarded as fresh fish. Fish has started to degrade when the TVC value exceeds 5.00 log CFU/g (Lund et al., 2000).

Our study showed storage time ($P < 0.001$) affected the TVC content (Fig. 4). When the storage commenced, the TVC of the fresh fish was 4.18 log cfu/ml. As storage time extended, the TVC count markedly increased by 15 days (5.42 log cfu/ml) and 23 days (6.32 log cfu/ml) but did not exceed the spoilage thresholds (10^8 – 10^9 cfu/g of specific spoilage bacteria) in iced fish stored aerobically (Gram & Huss, 1996). Similar result was obtained at day 14 (6.93 log cfu/g) by Baixas-Nogueras et al. (2003) from fresh and gutted hake (*M. merluccius*) stored in flaked ice and inside a refrigerator set at 4 °C. Also, present results agree with other authors who evaluated TVC content in other Family fish. Rodríguez et al. (2003), whose level after 14 and 22 days of storage was 6.47 and 6.36 log cfu/g, respectively, in farmed not headed or gutted specimens of turbot (*Psetta maxima*).

A study carried out by Fuentes-Amaya et al. (2016) found filleted fish market species, barramundi, Atlantic salmon, blue-spotted emperor, saddletail snapper, and crimson snapper storage at 4 °C in vacuum packaging, exceeding the spoilage thresholds at day 5. In this case, TVC levels changed from an average of 5.8 ± 0.3 log₁₀ cfu/g (day 1) to 7.2 ± 0.5 log₁₀ cfu/g (day 3), and 8.5 ± 0.8 log₁₀ cfu/g (day 5). All of them were found to be above the acceptability limits (6 log₁₀ cfu/g) in some Australian supermarkets and close to the microbiological limit for seafood of 7 log₁₀ cfu/g, according to International Commission on Microbiological Specification for Foods – ICMSF (ICMSF, 1986) after 3 days.

And Li et al. (2023) showed the total viable count of fresh large yellow croaker changed from 3.17 lg/(CFU/g) at day 0 to 7.43 lg/(CFU/g) on the 9th day, which exceeded the maximum acceptable level of the total viable count in marine fish as stipulated by the ICMSF (1986).

3.7. Comparative evaluation of organoleptically, chemical and microbiological analyses for retail fresh hake (*Merluccius australis*) during chilled storage

Chilled storage (<4 °C) of Chilean southern hake showed relatively rapid sensory deterioration, with key attributes reaching the unacceptable (rejection) category between days 5 (eyes) and 7 (eyes and gills), limiting the shelf-life based strictly on sensory criteria to about 4–6 days. In contrast, chemical spoilage indicators showed a slower progression; TMA-N reached critical values on day 9, which could cause digestive discomfort and adverse reactions in sensitive individuals, while TVB-N significantly exceeded legal limit of 30 mg/100 g after day 9 (closer to day 15), suggesting a risk of toxicity and possible digestive intolerance if the fish is consumed in poor condition.

The present study also considered the presence and accumulation of histamine as a possible indicator of scombroid intoxication. Fortunately, the presence of histamine was only detected in 4.5 % of the hake samples on day 15, and its value remained below the permitted limits (100–200

mg/kg). However, these values could increase when the fish is not stored correctly, and could cause symptoms of histamine intoxication, such as reddening of the skin, headaches, nausea and vomiting, and respiratory problems in severe cases. On the other hand, a progressive increase in microbial load observed during storage did not reach critical levels of bacterial spoilage but could suggest a risk of secondary contamination. Also the presence of psychrotrophic microorganisms could generate thermally stable toxins that are difficult to eliminate even with cooking.

Present findings revealed a strong correlation between organoleptic changes and chemical spoilage markers, supporting the use of sensory cues as reliable freshness indicators. However, although sensory evaluation offers a more accessible tool for inspectors and consumers, sometimes it is difficult or unreliable (e.g., due to processing such as heading evisceration, practices that mask any signs of poor freshness, and the risk of mixed batches of fresh and aged fish being sold together which could trigger a significant health risk).

On the other hand, although not reflecting the earliest signs of sensory rejection, both TVB-N and TMA proved to be reliable indicators of spoilage progression, showing consistent and statistically significant increases over time. Their usefulness lies in providing objective and quantifiable parameters that eventually cross established legal or commercial thresholds, serving as reference points for product refusal, particularly relevant for regulatory control or when sensory evaluation is difficult or unreliable.

This temporal discrepancy between sensory deterioration and the exceeding of chemical limits highlights a potential risk in the retail market, and relying solely on chemical limits might allow the sale of fish that already exhibit undesirable sensory characteristics for the consumer. So, integrating different strategies could improve consumer safety and product transparency in seafood markets. It is important to improve food assessment at points of sale, reinforce good practices throughout the chain (including the use of ice and strict temperature control), and increase information for producers and consumers.

Additionally, future research from interdisciplinary collaborations, could contribute to the development of advanced storage techniques tailored for fish and seafood products, such as the use of natural preservatives, edible coatings, and modified atmosphere packaging which have shown significant potential to extend the shelf-life and preserve the sensory and microbiological quality of fish species (Ólafsdóttir et al., 2007; Ghaly et al., 2010; Biswas et al., 2023; Kurek et al., 2024; Udana Eranda et al., 2024).

Other future research should consider implementing rigorously controlled experimental designs using freshly harvested hake obtained directly from artisanal or industrial fisheries to determine the maximum shelf-life of this species under ideal postharvest conditions. Immediate processing and standardized cold storage protocols would allow for the establishment of reliable baseline quality parameters and enable a more precise evaluation of spoilage progression and shelf-life under optimal conditions.

Nevertheless, present results provide valuable insights into the typical and real quality of Chilean southern hake available to consumers in Valdivia retail market, located in southern Chile. Despite the study's limitations, this work provides the first empirical evidence on the evolution of these indicators in southern hake under these refrigerated storage conditions common in southern Chile, highlighting the need to ensure the quality and safety of this important fishery resource; and, indirectly assessing the potential health hazards related to organoleptic evaluations, chemical indicators, as harmful substances (e.g., histamine), and microbial analysis.

4. Conclusions

The present study offers relevant insights into the retail-level quality and shelf-life of Chilean southern hake (*Merluccius australis*). First, the elevated initial pH values observed suggest that the specimens may have undergone partial deterioration prior to acquisition in the retail market.

The storage of Chilean southern hake specimens in chilled storage at temperature lower than 4 °C after its acquisition, allowed maintenance of quality and extended shelf-life up to day 4, according to sensory analysis used to international commercial fishes.

For national market quality and extended shelf-life up to day 8, according to TMA-N contents that are related to fish spoilage, confirming the deterioration of the fish, and reinforcing the need for stricter monitoring during storage; and, before day 15 to TVB-N levels, used as an index for freshness of fish. Both could be considered reliable indicators by freshness in Chilean southern hake at present scenario. To the best of our knowledge the present research is the first empirical study in *M. australis* that finds TVB-N and TMA-N levels increased significantly after 7 days of storage, in both cases; and exceeded the allowable limit to the family *Merlucciidae* at day 15 and 9, respectively.

Moreover, the results obtained showed variability within each day of analysis which could indicate not adequate commercial practices in the retail market in Valdivia when mixing fish from different days and batches to sell them. Additionally, in the retail market fish can be found without heads and sensory evaluation would not be an accessible and reliable method for assessing freshness in these cases; practices that mask any signs of poor freshness, which could trigger a significant health risk.

So, TVB-N and TMA-N reference methods would be recommended to refuse southern hake in markets.

Apart from that, it will be necessary to take into consideration the limitations of the model and to increase the number of samples in future work. Also, it was not possible to make repeated measurements on the same fish during experimental time, considering the different analyses performed at each storage time on each. However, it did not affect the results of the study, because results were considered at the population level.

Hence urgent measures that should be carried out are: (i) increase food assessment, and (ii) offer more information about good practice in food chain to producers, fishermen, and consumers, and to use slurry or flake ice batch together with refrigeration chamber to better maintenance of quality and extended shelf-life of chilled fish products in the storage of fish specimens.

Ethical statement

The authors of the manuscript entitled “**A comprehensive approach to assessment of the quality and shelf-life for retail fresh hake (*Merluccius australis*) during Chilled Storage**”, declare:

This research includes sensory evaluation and the panelists that assessed organoleptically the freshness of fish are part of the staff of Food and Water Laboratory of Austral University of Chile.

The study complies with all Chilean ethical regulations and all experiments were conducted according to established ethical guidelines; however, this kind of sensory test in Chile does not require ethical approval by an Ethics Committee.

They also confirm that this is an original work; it has never been published, nor is it currently under consideration for publication elsewhere.

CRedit authorship contribution statement

Carmen Lopez-Joven: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Reydoret Umaña:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Investigation. **Paola Guajardo:** Methodology, Investigation. **Ignacio de Blas:** Writing – review & editing, Writing – original draft, Visualization, Validation, Formal analysis, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. They also confirm that this is an original work; it has never been published, nor is it currently under consideration for publication elsewhere.

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Data availability

Data will be made available on request.

References

- Ali, M. Y., Sharif, M. I., Adhikari, R. K., & Faruque, O. (2010). Post mortem variation in total volatile base nitrogen and trimethylamine nitrogen between Galda (*Macrobrachium rosenbergii*) and Bagda (*Penaeus monodon*). *University Journal of Zoology, Rajshahi University*, 28, 7–10. <https://doi.org/10.3329/ujzr.v28i0.5278>
- Baixas-Nogueiras, S., Bover-Cid, S., Veciana-Nogueu, T., Nunes, M. L., & Vidal-Carou, M. C. (2003). Development of a quality index method to evaluate freshness in Mediterranean hake (*Merluccius merluccius*). *Journal of Food Science*, 68, 1067–1071. <https://doi.org/10.1111/j.1365-2621.2003.tb08289.x>
- Biswas, O., Kandasamy, P., Nanda, P. K., Biswas, S., Lorenzo, J. M., Das, A., Alessandrini, L., Lamri, M., Das, A. K., & Gagaoua, M. (2023). Phytochemicals as natural additives for quality preservation and improvement of muscle foods: A focus on fish and fish products. *Food Materials Research*, 3, 5. <https://doi.org/10.48130/FMR-2023-0005>
- Biji, K. B., Ravishankar, C. N., Venkateswarlu, R., Mohan, C. O., & Srinivasa Gopal, T. K. (2016). Biogenic amines in seafood: A review. *Journal of Food Science and Technology*, 53, 2210–2218. <https://doi.org/10.1007/s13197-016-2224-x>
- Davidek, T., & Davidek, J. (1995). Biogenic amines. In Davidek, J. (Ed), *Natural toxic compounds of foods*, (pp. 108–123). CRC Press Boca Roton.
- Erandi, D. H. U., Chaijan, M., Uysal-Unalan, I., Panpipat, W., Naik, A. S., Dib, A. L., Karnjanapratum, S., & Gagaoua, M. (2024). Biopreservation of pre-processed fresh fish by bio-based coatings: A single strategy with multiple benefits towards waste prevention. *Food Bioscience*, 58(4), Article 103696. <https://doi.org/10.1016/j.fbio.2024.103696>
- European Commission (EC). (1976). Council Regulation (EEC) No 103/76 of 19 January 1976 laying down common marketing standards for certain fresh or chilled fish. *Official Journal of the European Communities*, L 20 28.01.1976, p. 29–34, ELI: <http://data.europa.eu/eli/reg/1976/103/oj>.
- European Commission (EC). (1991). Council Directive 91/493/EEC of 22 July 1991 laying down the health conditions for the production and the placing on the market of fishery products. *Official Journal of the European Communities*, L 268, 24.09.1991, p. 15–34. ELI: <http://data.europa.eu/eli/dir/1991/493/oj>.
- European Commission (EC). (2005a). Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. *Official Journal of the European Union*, L 338, 22.12.2005, p. 1–26. Retrieved from <http://data.europa.eu/eli/reg/2005/2073/oj>.
- European Commission (EC). (2005b). Commission Regulation (EC) No 2074/2005 of 5 December 2005 laying down implementing measures for certain products under Regulation (EC) No 853/2004 of the European Parliament and of the Council and for the organisation of official controls under Regulation (EC) No 854/2004 of the European Parliament and of the Council and Regulation (EC) No 882/2004 of the European Parliament and of the Council, derogating from Regulation (EC) No 852/2004 of the European Parliament and of the Council and amending regulations (EC) No 853/2004 and (EC) No 854/2004. *Official Journal of the European Union*, L 338/27, 22.12.2005, p. 1–33. Retrieved from <https://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:32005R2074>.
- Directive 1022/2008/EC (EC, 2008) Commission Regulation (EC) No 1022/2008 of 17 October 2008 amending Regulation (EC) No 2074/2005 as regards the total volatile basic nitrogen (TVB-N) limits. Retrieved from <https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex:32008R1022>.
- Fuentes-Amaya, L. F., Munyard, S., Fernandez-Piquer, J., & Howieson, J. (2016). Sensory, microbiological and chemical changes in vacuum-packaged blue spotted emperor (*Lethrinus* sp), saddlelet snapper (*Lutjanus malabaricus*), crimson snapper (*Lutjanus erythroterus*), barramundi (*Lates calcarifer*) and atlantic salmon (*Salmo salar*) fillets stored at 4°C. *Food Science & Nutrition*, 4, 479–489. <https://doi.org/10.1002/fsn3.309>
- García, M. R., Vilas, C., Herrera, J. R., Bernárdez, M., Balsa-Canto, E., & Alonso, A. A. (2015). Quality and shelf-life prediction for retail fresh hake (*Merluccius merluccius*). *International Journal of Food Microbiology*, 208, 65–74. <https://doi.org/10.1016/j.ijfoodmicro.2015.05.012>

- Ghaly, A. E., Dave, D., Budge, S., & Brooks, M. S. (2010). Fish spoilage mechanisms and preservation techniques: Review. *American Journal of Applied Sciences*, 7(7), 859–877. <https://doi.org/10.3844/ajassp.2010.859.877>
- Gram, L., & Huss, H. H. (1996). Microbiological spoilage of fish and fish products. *International Journal of Food Microbiology*, 33, 121–137. [https://doi.org/10.1016/0168-1605\(96\)01134-8](https://doi.org/10.1016/0168-1605(96)01134-8)
- Hebard, C.E., Flick, G.J., & Martin, R.E. (1982). Occurrence and significance of trimethylamine oxide and its derivatives in fish and shellfish. In Martin, R., Flick, G., Hebard, C. E. & Ward, D.R. (Eds.), *Chemistry and biochemistry of marine food products* (pp. 149–272). AVI Publishing Co., Westport, CT.
- Horsfall, M. Jr., Kinigoma, B. S., & Spiff, A. I. (2006). Evaluation of the levels of total volatile bases and trimethylamine formed in fish stored at low temperature. *Bulletin of the Chemical Society of Ethiopia*, 20(1), 155–159.
- Horsfall Jr, M., Gentleman, P. C., Adowei, P., & Dikio, E. D. (2023). Evaluation of total volatile bases and trimethylamine in hake (*Merluccius capensis*) fish preserved at low temperature in Vanderbijlpark, South Africa. *World Journal of Advanced Research and Reviews*, 19, 1082–1092. <https://doi.org/10.30574/wjarr.2023.19.1.1472>
- Hungerford, J. M. (2010). Scombroid poisoning: A review. *Toxicon: Official Journal of the International Society on Toxinology*, 56, 231–243. <https://doi.org/10.1016/j.toxicon.2010.02.006>
- ICMSF. (1986). *International commission on microbiological specification for foods. microorganisms in foods 2. Sampling for microbiological analysis: Principles and specific applications*. Toronto, ON, Canada: University of Toronto. Press.
- Instituto Nacional de Normalización (INN). (2018). NCh2668:2018. Productos hidrobiológicos- determinación de nitrógeno básico volátil total (NBVT). Retrieved from <https://ecommerce.inn.cl/nch2668201862889>.
- Instituto Nacional de Normalización (INN). (2001). NCh2637:2001. Productos hidrobiológicos- determinación de histaminas y otras aminas biógenas- Método HPLC con detector UV. Retrieved from <https://ecommerce.inn.cl/nch2637200144424>.
- Instituto Nacional de Normalización (INN). (2002a). NCh2738:2002. Productos hidrobiológicos- Medición de pH. Retrieved from <https://ecommerce.inn.cl/nch2738200244564>.
- Instituto Nacional de Normalización (INN). (2002b). NCh2757:2002. Productos hidrobiológicos- Determinación de nitrógeno de trimetilamina. Retrieved from <https://ecommerce.inn.cl/nch2757200244591>.
- Instituto Nacional de Normalización (INN). (2002c). NCh2659:2002. Productos hidrobiológicos - Determinación de microorganismos aerobios mesófilos - Técnica de recuento en placa a 35°C. Retrieved from <https://ecommerce.inn.cl/nch2659200244446>.
- Jouki, M., Yazdi, F. T., Mortazavi, S. A., Koocheki, A., & Khazaei, N. (2014). Effect of quince seed mucilage edible films incorporated with oregano or thyme essential oil on shelf life extension of refrigerated rainbow trout fillets. *International Journal of Food Microbiology*, 174, 88–97. <http://doi/10.1016/j.ijfoodmicro.2014.01.001>.
- Karovičová, J., & Kohajdová, Z. (2005). Biogenic amines in food. *Chemical Papers*, 59, 70–79.
- Khoshnoudi-Nia, S., & Moosavi-Nasab, M. (2019). Prediction of various freshness indicators in fish fillets by one multispectral imaging system. *Scientific Reports*, 9. <https://doi.org/10.1038/s41598-019-51264-z>. Article 14704.
- Kurek, M., Pišonić, P., Šćetar, M., Jančić, T., Canak, I., Vidaček Filipec, S., Benbettaieb, N., Debeaufort, F., & Galić, K. (2024). Edible coatings for fish preservation: Literature data on storage temperature, product requirements, antioxidant activity, and coating performance-A review. *Antioxidants*, 13(11), 1417. <https://doi.org/10.3390/antiox13111417>
- Li, B., Liu, S., Chen, X., Su, Y., Pan, N., Liao, D., ... Liu, Z. (2023). Dynamic changes in the microbial composition and spoilage characteristics of refrigerated large yellow croaker (*Larimichthys crocea*) during storage. *Foods (Basel, Switzerland)*, 12(21), 3994. <https://doi/10.3390/foods12213994>.
- Lund, B. M., Parker, T. C., & Gould, G. W. (2000). The microbiological safety and quality of food. *Toxicogenic Fungi Mycotoxins*, 57, 209–215.
- McLauchlin, J., Little, C. L., Grant, K. A., & Mithani, V. (2005). Scombrototoxic fish poisoning. *Journal of Public Health*, 28, 61–62. <https://doi/10.1093/pubmed/fdi063>.
- Ministerio de Salud (MINSAL). (2019). Reglamento Sanitario de los Alimentos. Decreto N° 977/96 actualizado. Retrieved from http://www.dinta.cl/wp-content/uploads/2019/03/RSA-DECRETO_977_96_act_enero-2019_DINTA_.pdf.
- Moral, A. (1987). Métodos físico-químicos de control de calidad de pescados. *Alimentación, Equipos y Tecnología*, 115–122, 5-6.
- Ólafsdóttir, G., Martinsdóttir, E., Oehlenschläger, J., Dalgaard, P., Jensen, B., Undeland, I., Mackie, I. M., Henehan, G., Nielsen, J., & Nilsen, H. (2007). Methods to evaluate fish freshness in research and industry. *Trends in Food Science & Technology*, 8, 258–265. [https://doi.org/10.1016/S0924-2244\(97\)01049-2](https://doi.org/10.1016/S0924-2244(97)01049-2)
- Özogul, A., Özogul, F., Kuley, E., Özkutuk, A. S., Gokbulut, C., & Kose, S. (2006). Biochemical, sensory and microbiological attributes of wild turbot (*Scophthalmus maximus*), from the Black Sea, during chilled storage. *Food Chemistry*, 99, 752–758. <https://doi.org/10.1016/j.foodchem.2005.08.053>
- Pedrosa-Menabrito, A., & Regenstein, J. M. (1988). Shelf-life extension of fresh fish – A review part I - spoilage of fish. *Journal of Food Quality*, 11, 117–127. <https://doi.org/10.1111/j.1745-4557.1988.tb00872.x>
- Pedrosa-Menabrito, A., & Regenstein, J. M. (1990). Shelf-life extension of fresh fish – A review part III - fish quality and methods of assessment. *Journal of Food Quality*, 13 (3), 209–223. <https://doi.org/10.1111/j.1745-4557.1990.tb00018.x>
- Perez-Villarreal, B., & Howgate, P. (1987). Spoilage of European hake (*Merluccius merluccius*) in ice. *Journal of the Science of food and Agriculture*, 41, 335–350. <https://doi.org/10.1002/jsfa.2740410406>
- Pérez-Villarreal, B., & Pozo, R. (1990). Chemical composition and ice spoilage of albacore (*Thunnus alalunga*). *Journal of Food Science*, 55, 678–682. <https://doi.org/10.1111/j.1365-2621.1990.tb05205.x>
- Rodríguez, O., Barros-Velázquez, J., Ojea, A., Piñeiro, C., & Aubourg, S. P. (2003). Evaluation of sensory and microbiological changes and identification of proteolytic bacteria during the iced storage of farmed turbot (*Psetta maxima*). *Journal of Food Science*, 68, 2764–2771. <https://doi.org/10.1111/j.1365-2621.2003.tb05802.x>
- Rodríguez, O., Losada, V., Aubourg, S. P., & Barros-Velázquez, J. (2004). Enhanced shelf-life of chilled European hake (*Merluccius merluccius*) stored in slurry ice as determined by sensory analysis and assessment of microbiological activity. *Food Research International*, 37, 749–757. <https://doi.org/10.1016/j.foodres.2004.03.008>
- Ruiz-Capillas, C., & Moral, A. (2001). Correlation between biochemical and sensory quality indices in hake stored in ice. *Food Research International*, 34, 441–447. [https://doi.org/10.1016/S0963-9969\(00\)00189-7](https://doi.org/10.1016/S0963-9969(00)00189-7)
- Servicio Nacional de Pesca y Acuicultura (SERNAPESCA). (2021). Anuarios Estadísticos de Pesca y Acuicultura. Retrieved from <http://www.sernapesca.cl/informacion-utilidad/anuarios-estadisticos-de-pesca-y-acuicultura>.
- Servicio Nacional de Pesca y Acuicultura (SERNAPESCA). (2023). Safety and Certification Manual. Parte II: Sección III. Control de Exportación y Certificación. Capítulo IV: Requisitos para autorización de embarque y certificación sanitaria. Pp 29-30. Retrieved from http://www.sernapesca.cl/sites/default/files/parte_ii_seccion_iii_control_de_exportacion_y_certificacion_v20230208.pdf.
- Shewan, J.M. (1976): The bacteriology of fresh and spoiling fish and biochemical changes induced by bacterial action. In: Proc. of the Conf. on the handling, processing and marketing of tropical fish. London, Tropical Products Institute, pp. 377–94.
- Sotelo, C. G., Gallardo, J. M., Piñeiro, C., & Pérez-Martin, R. (1995). Trimethylamine oxide and derived compounds' changes during frozen storage of hake (*Merluccius merluccius*). *Food Chemistry*, 53, 61–65. [https://doi.org/10.1016/0308-8146\(95\)95787-7](https://doi.org/10.1016/0308-8146(95)95787-7)
- Stansby, M.E., Harrison, R.W., Dassow, J. & Sater, M. (1944). Spoilage of tropical fish and product development. (Reilly, A. ed.) Food and Agricultural Organization of the United Nations (Rome). p.122.
- Taliadourou, D., Papadopoulos, V., Domvridou, E., Savvaidis, I. N., & Kontominas, M. G. (2003). Microbiological, chemical and sensory changes of whole and filleted Mediterranean aquacultured sea bass (*Dicentrarchus labrax*) stored in ice. *Journal of the Science of Food and Agriculture*, 83, 1373–1379. <https://doi.org/10.1002/jsfa.1553>
- Venugopal, V. (2002). Biosensors in fish production and quality control. *Biosensors and Bioelectronics*, 17, 147–157. [https://doi.org/10.1016/S0956-5663\(01\)00180-4](https://doi.org/10.1016/S0956-5663(01)00180-4)
- Zheng, R., Xu, X., Xing, J., Cheng, H., Zhang, S., Shen, J., & Li, H. (2020). Quality evaluation and characterization of specific spoilage organisms of Spanish mackerel by high-throughput sequencing during 0°C cold chain logistics. *Foods (Basel, Switzerland)*, 9(3), 312. <https://doi.org/10.3390/foods9030312>