Research Article

Effect of high-pressure treatment on microbial activity and lipid oxidation in chilled coho salmon

Santiago P. Aubourg[1], Gipsy Tabilo-Munizaga[2], Juan E. Reyes[2], Alicia Rodríguez[3], Mario Pérez-Won[4]

[1] Food Technology Department, Instituto de Investigaciones Marinas (CSIC), Vigo, Spain

[2] Food Engineering Department, Universidad de Bío-Bío, Chillán, Chile

[3] Food Science and Chemical Technology Department, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile

[4] Food Engineering Department, Universidad de La Serena, La Serena, Chile

This work studies the effect of a previous hydrostatic high-pressure (HHP) treatment on chilled farmed coho salmon (*Oncorhynchus kisutch*). Three different HHP conditions were applied (135 MPa-30 s, 170 MPa-30 s, and 200 MPa-30 s for treatments T-1, T-2, and T-3, respectively) and compared to untreated (control) fish throughout a 20-day chilled storage. Microbial activity and lipid oxidation development were analyzed. Assessment of aerobe, psychrotroph, *Shewanella* spp. and *Pseudomonas* spp. counts and trimethylamine formation showed a marked inhibitory effect ($p < 0.05$) of HHP treatment on microbial activity, with this effect increasing with the pressure value employed. Related to lipid oxidation development, higher peroxide mean values (day 10–20 period) were found in control samples and fish treated under T-1 condition when compared to their counterparts corresponding to T-2 and T-3 treatments. On the contrary, quantification of thiobarbituric acid-reactive substances and fluorescent interaction compounds showed higher levels ($p < 0.05$) in fish samples corresponding to T-2 and T-3 treatments. In spite of the lipid oxidation development found, polyene index and tocopherol isomer (α and γ) content did not provide differences ($p > 0.05$) as a result of previous HHP treatment.

Practical applications

This study focuses on a fish species (coho salmon; *Oncorhynchus kisutch*) that has recently received great attention because of its increasing farming production and availability to elaborate different kinds of commercial products. Previous research has shown quality losses during its traditional ice chilling. In the present study, the effect of a previous hydrostatic high-pressure (HHP) treatment was tested and compared to untreated fish during a 20-day chilled storage. A comparative study of the microbial activity and lipid oxidation was performed. As a result of the two highest pressure condi-

tions tested, microbial activity was partially inhibited in chilled fish, while a higher secondary and tertiary lipid oxidation compound formation was observed. However, such rancidity development did not lead to significant changes in PUFA and tocopherol isomer contents. It is concluded that previous employment of HHP conditions can provide a quality and safety enhancement during the chilled storage of this farmed species.

1 Introduction

Fish species are known to deteriorate rapidly postmortem. To slow down the mechanisms involved in quality loss, the fish should be refrigerated immediately after capture. Flake ice has been the most often employed method to cool and store fish products and partially inhibits detrimental effects on the commercial value. In spite of such efforts, significant dete-

Correspondence: Santiago P. Aubourg, Food Technology Department, Instituto de Investigaciones Marinas (CSIC), Eduardo Cabello, 6, 36308 Vigo, Spain.
E-mail: saubourg@iim.csic.es
Fax: +34-986-292762

rioration of sensory quality and nutritional value has been detected in chilled fish as a result of different damage pathways, such as endogenous enzymatic activity, microbial development and lipid oxidation [1, 2].

As a result of an increasing consumer demand for high-quality fresh products, fish technologists and the fish trade have developed different advanced processing systems. Among them, hydrostatic high-pressure (HHP) technology has been shown to maintain sensory and nutritional properties, while inactivating microbial development and leading to a shelf life extension and a safety enhancement [3, 4]. This technology has shown potential application in the seafood industry for the surimi and kamaboko production [5, 6], as assisting thawing [7] and for the cold-smoked fish preparation [8].

An additional positive effect of HHP treatment is that deteriorative molecules, such as hydrolytic and oxidative endogenous enzymes, can be inactivated for a further storage/processing of the fish product [3, 4]. However, HHP has been reported to damage membranes, denature proteins and cause changes in cell morphology; although covalent bonds are not broken, weak energy bonds like hydrogen and hydrophobic bonds can be irreversibly modified, leading to important consequences for the secondary, tertiary and quaternary structures in proteins [9–11]. In addition, HHP treatment has been reported to induce oxidative changes in lipid matter of fish products, so that an important loss of rancidity stability has been mentioned [3, 12].

Among cultivated fish, coho salmon (*Oncorhynchus kisutch*), also called silver salmon, has received great attention because of its increasing production in countries like Chile, Japan and Canada [13], in parallel to important capture production in countries such as USA, Russian Federation, Canada and Japan [14]. Previous research related to the chilled storage of this species accounts for the development of different spoilage pathways and quality loss [15, 16]. In the present work, the effect of a previous HHP treatment on chilled storage of this species was investigated. The study focuses on the microbial activity and the lipid oxidation development.

2 Materials and methods

2.1 Raw fish, processing and sampling

Coho salmon specimens (50–52 cm length; 2.8–3.0 kg weight) were obtained from an aquaculture facility (Aquachile, S. A., Puerto Montt, X Región, Chile). Individuals were sacrificed in the plant by a sharp blow to the head, the gills cut, bled in a water-ice mixture, headed, gutted and transported to the laboratory during 24 h under slurry ice conditions (40% ice and 60% water; -1.0 °C) at a 1 : 1 fish-to-ice ratio. Then, the fish was filleted, cut into pieces (weight range: 125–150 g) and placed in flexible polyethylene bags.

HHP treatment was performed in a cylindrical loading container at room temperature in a 2-L pilot high-pressure unit (Avure Technologies Incorporated, Kent, WA, USA) using water as the pressurizing medium. Three different HHP conditions (135 MPa for 30 s, 170 MPa for 30 s and 200 MPa for 30 s: treatments T-1, T-2 and T-3, respectively) were applied to fish and compared to untreated fish (control, treatment C). Fish was then kept under chilling conditions (traditional flake ice) in a refrigerated room (4 °C). Sampling was carried out on salmon white muscle at days 0, 6, 10, 15 and 20 of chilled storage. For all kinds of samples, three different batches ($n = 3$) were considered and analyzed separately.

A different response to HHP treatment has been reported to occur according to different factors in marine species and products such as species nature, chemical composition and size [4, 17]. Accordingly, a preliminary study was undertaken before choosing the HHP treatment range to be applied in the present experiment. Then, two independent variables were considered (pressure to be applied and holding time) and their effect on visual analysis of salmon fish (color, gaping, elasticity and firmness) was carried out. Pressure and holding time conditions corresponding to the best visual appearance obtained were selected for the actual research. Such HHP conditions are in agreement with the optimized conditions previously recommended for farmed turbot (*Scophthalmus maximus*) fillets as not contributing to important physico-chemical modifications [18].

2.2 Microbial analyses

All samples were analyzed for counts of aerobic mesophilic and psychrotrophic microorganisms, *Pseudomonas* spp. and H_2S-producing bacteria (*Shewanella* spp.). Of each sample, 10 g was obtained aseptically and homogenized with 90 mL of chilled maximum recovery diluent (Oxoid, Basingstoke, UK) for 60 s. Further, decimal dilutions were made with the same diluent and duplicates of at least three dilutions were plated on the appropriate media, according to the following procedures.

In order to enumerate the aerobic mesophilic and psychrotrophic microorganisms, 1 mL of each dilution were pour-plated in Long and Hammer's medium with 1% NaCl, as described by Van Spreekens [19]. After incubation at 30 °C for 72 h (for mesophilic counts) and at 7 °C for 10 days (for psychrotrophic counts), plates with 30–300 colonies were counted.

To count the *Pseudomonas* spp., 0.1 mL of each dilution were spread on the surface of *Pseudomonas* CFC-selective medium (Oxoid). After incubation at 25 °C for 2 days, plates with 30–300 colonies were counted and five colonies with different morphological aspects were purified on Trypticase Soy Agar (TSA; Difco, Detroit, USA), and further confirmed to be isolates of *Pseudomonas* spp. by checking for production of cytochrome oxidase [20] and the ability to utilize glucose in the oxidation-fermentation test [21]. Results on total counts of

Pseudomonas spp. were based on the percentage of colonies tested that were identified as *Pseudomonas* spp.

For the H_2S-producing bacteria count, 1 mL of dilution was inoculated into 10 mL Iron Agar Lingby (Oxoid) and, after mixing and solidifying, each plate was covered with a layer of the same medium. After incubation at 25 °C for 3 days, plates with 15–150 characteristic colonies (black colonies due to precipitation of FeS) were counted and five randomly selected typical colonies were purified on TSA, and further confirmed by establishing the following morphological and biochemical properties as described by Gram et al. [22]: gram negative, motile rods with positive catalase and oxidase reactions, oxidative glucose metabolism, and H_2S producing.

Microbiological data were transformed into logarithms of the number of colony-forming units (CFU/g muscle).

2.3 Chemical analyses

Total volatile base-nitrogen (TVB-N) values were measured by a distillation-titration method, according to the Aubourg et al. [23] method: Fish muscle (10 g) was extracted with 6% perchloric acid and brought up to 50 mL. Then, steam distillation of the acid extracts rendered alkaline to pH 13 with 20% NaOH was carried out. Finally, the TVB-N content was determined by titration of the distillate with 10 mM HCl. The results were expressed as mg TVB-N/kg muscle.

Trimethylamine-nitrogen (TMA-N) values were determined by the picrate method, as previously described by Tozawa et al. [24]. This technique involves the preparation of a 5% trichloroacetic acid extract of fish muscle (10 g/25 mL). The results were expressed as mg TMA-N/kg muscle.

Moisture content was determined by the difference between the weight of fresh homogenized muscle (1–2 g) and the weight recorded after 4 h at 105 °C, according to the AOAC method [25]. Results were expressed as g water/kg muscle.

Lipids were extracted by the Bligh and Dyer [26] method, by employing a single-phase solubilization of the lipids using a chloroform/methanol (1 : 1) mixture. Quantification results were expressed as g lipid/kg muscle.

The peroxide value (PV) was determined by peroxide reduction with ferric thiocyanate, according to the Chapman and McKay [27] method. Results were expressed as meq active oxygen/kg lipids.

The thiobarbituric acid index (TBA-i) was determined according to Vyncke [28]. This method is based on the reaction between a trichloroacetic acid extract of the fish muscle and thiobarbituric acid. The content of thiobarbituric acid-reactive substances (TBARS) was spectrophotometrically measured at 532 nm and the results were expressed as mg malondialdehyde/kg muscle.

The formation of fluorescent compounds was determined by measurements at 393/463 nm and 327/415 nm as described by Aubourg et al. [29]. The relative fluorescence (RF) was calculated as follows: $RF = F/F_{st}$, where F is the fluores-

cence measured at each excitation/emission maximum, and F_{st} is the fluorescence intensity of a quinine sulfate solution (1 μg/mL in 0.05 M H_2SO_4) at the corresponding wavelength. The fluorescence ratio (FR) was calculated as the ratio between the two RF values: $FR = RF_{393/463 \, nm}/RF_{327/415 \, nm}$. The FR value was determined in the aqueous phase resulting from the lipid extraction [26].

Lipid extracts were converted into fatty acid methyl esters (FAME) by employing acetyl chloride and then analyzed by gas chromatography, according to Aubourg et al. [29]. FAME were analyzed by means of a Perkin-Elmer 8700 chromatograph employing a fused-silica capillary column SP-2330 (0.25 mm i.d. × 30 m; Supelco, Bellefonte, PA, USA). Nitrogen at 10 psi as carrier gas and a flame ionization detector (FID) at 250 °C were used. Peaks corresponding to fatty acids were identified by comparison of their retention times with standard mixtures (Qualmix Fish, Larodan, Malmo, Sweden; FAME Mix, Supelco). Peak areas were automatically integrated, with 19:0 fatty acid being used as internal standard for quantitative analysis. The polyene index (PI) was calculated as the following fatty acid ratio: $(20:5n\text{-}3 + 22:6n\text{-}3)/16:0$.

Tocopherols were analyzed according to Cabrini et al. [30]: Lipophilic antioxidants were extracted from the muscle with hexane, brought to dryness under nitrogen flux, dissolved in isopropanol and injected for HPLC analysis. An ultrasphere ODS column (15 cm × 0.46 cm i.d.) was employed, by applying a gradient from 0 to 50% isopropanol. The flow rate was 1.5 mL/min. Detection was achieved at 280 nm. α-, γ- and δ-isomers were detected in farmed salmon samples, with their contents being expressed as mg/kg muscle.

2.4 Statistical analysis

Data ($n = 3$) obtained from the different microbial and chemical analyses were subjected to the ANOVA method ($p < 0.05$) to explore differences in two different ways: high-pressure effect and chilled storage effect (Statsoft, Statistica, version 6.0, 2001); comparison of means was performed using a least-squares difference (LSD) method. Correlation analysis among parameters (chilling time, microbial counts and chemical indices) was also carried out.

3 Results and discussion

3.1 Analysis of microbial activity by microbial and chemical parameter assessment

Total aerobe (Fig. 1) and psychrotrophic (Fig. 2) counts showed increasing ($p < 0.05$) values throughout the chilled storage for all fish samples, with this increase being lower in the last period of the experiment (days 15–20); accordingly, a good logarithmic fitting was obtained with chilling time in all
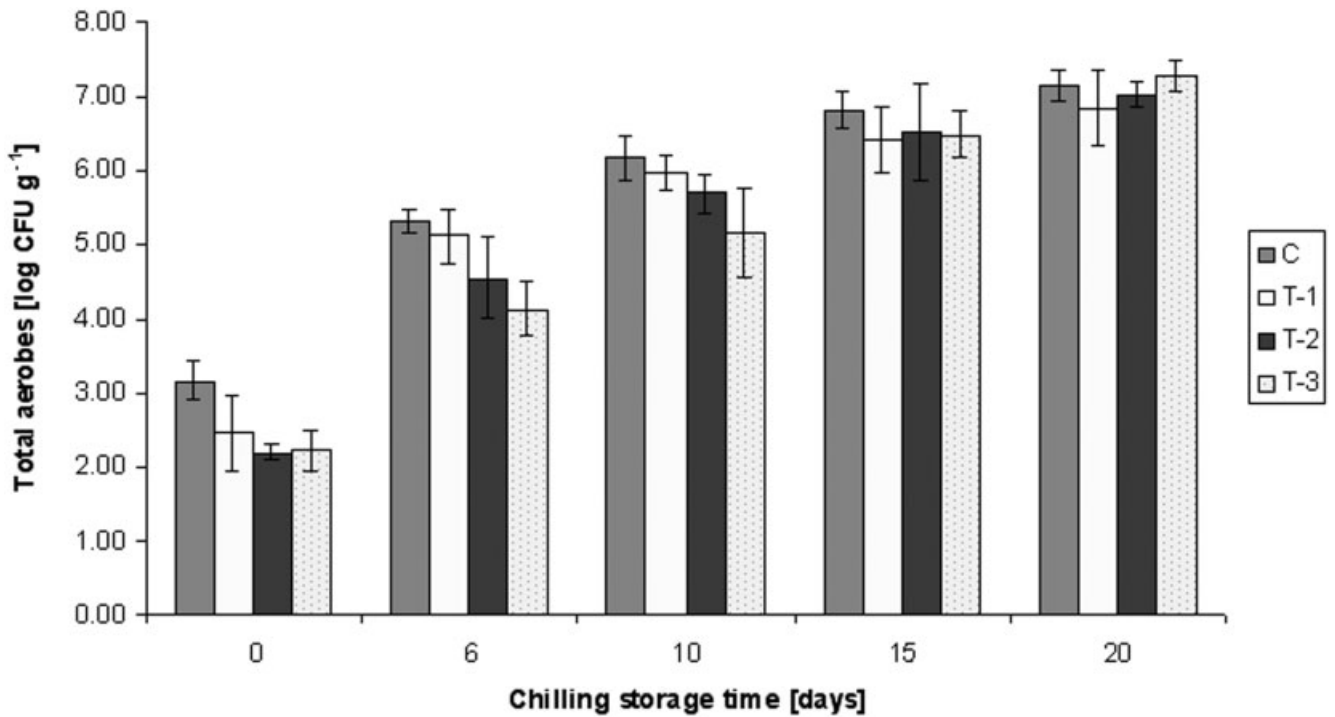
Figure 1. Comparative aerobe count assessment in chilled salmon previously treated under different HHP conditions. Mean values of three (*n* = 3) independent determinations are given. Standard deviations are denoted by bars. Treatment abbreviations: control (C), 135 MPa for 30 s (T-1), 170 MPa for 30 s (T-2), and 200 MPa for 30 s (T-3).
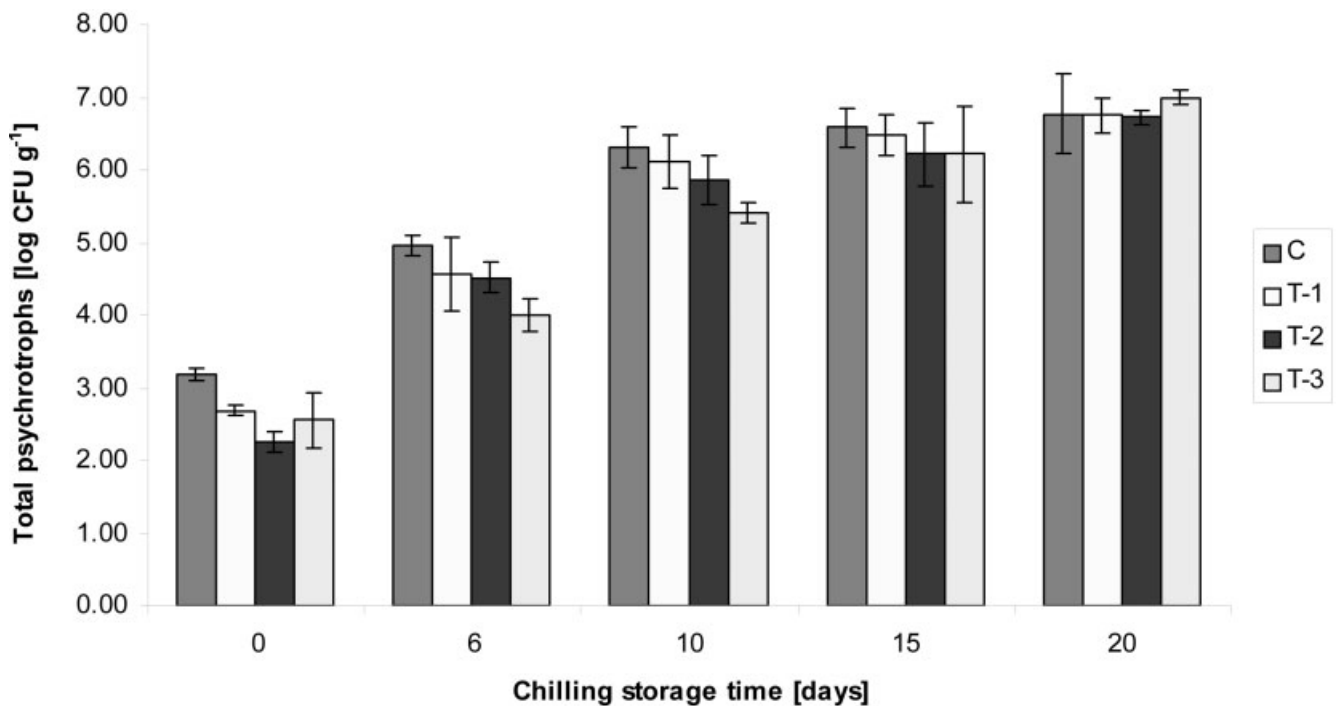

Figure 2. Comparative psychrotroph count assessment in chilled salmon previously treated under different HHP conditions. Mean values of three (*n* = 3) independent determinations are given. Standard deviations are denoted by bars. Treatment abbreviations as given in Fig. 1.

kinds of samples for both microbial group counts ($r^2 = 0.93$–0.94). Comparison of data corresponding to day 0 (Figs. 1, 2) showed a higher ($p < 0.05$) value in control fish when compared to fish treated under T-2 and T-3 conditions, so that an inhibitory effect on microbial values could be concluded according to previous research on different kinds of marine species and products [11, 17, 31, 32]. At this time (day 0), no differences ($p > 0.05$) could be assessed among samples corresponding to the different HHP conditions. When the chilling time is considered (day 6–10 period), a progressive mean microbial count decrease could be assumed as a result of increasing the pressure applied according to previous research [11, 17, 33]; indeed, higher aerobe and psychrotrophic values ($p < 0.05$) were present in control fish when compared to fish from T-2 and T-3 conditions. At the end of the experiment, all kinds of samples showed aerobe and psychrotrophic counts around 7.0 log CFU/g muscle, which is the acceptable microbial limit in fresh and frozen fish (The International Commission on Microbiological Specifications for Foods). In this sense, sensory rejection of fish products has typically been recognized at the 7.0–8.0 log CFU/g muscle range [2]. Such lack of difference at the end of the experiment is in agreement with previous research [33]: Sea bass fillets were treated under different HHP conditions (from 100 to 500 MPa) and then kept chilled; when an advanced stage of deterioration was attained (day 14), no differences among the different HHP-treated samples were observed in the microbial counts [33].

Microbial development analysis was complemented by the count assessment of *Shewanella* spp. and *Pseudomonas* spp., two known gram-negative microbial groups. Both kinds of microorganisms (Table 1) showed an important increase ($p < 0.05$) with time in all kinds of samples, with this increase being specially marked at day 6. As for the aerobe and psychrotrophic counts, good logarithmic fittings were obtained for all kinds of samples with chilling time ($r^2 = 0.90$–0.94). An inhibitory effect of HHP treatment could be assessed, since no presence of both spoilage groups could be detected in high-pressure-treated fish at day 0, independently of the HHP conditions applied. It is concluded that all pressure-time conditions tested in the present experiment were valuable in order to eliminate the initial loads of *Shewanella* spp. and *Pseudomonas* spp. During the last storage period (days 15–20), comparison among treatments showed higher ($p < 0.05$) levels in control samples than in pressure-treated fish for *Shewanella* spp. counts; additionally, an important partial inhibitory effect of the pressure value could be observed for the same period, so that fish corresponding to the T-1 treatment showed higher ($p < 0.05$) levels than their counterparts from the T-2 and T-3 conditions. Related to the presence of the *Pseudomonas* genus, comparison among fish samples did not provide clear tendencies throughout the day 6–20 period; however, higher mean values were obtained in most cases for samples corresponding to control fish during the day 0–15 period. At the end of the experiment, no differences ($p > 0.05$) were observed among samples.

Fish and shellfish are known to be generally spoiled by gram-negative bacteria. Previous research has shown that gram-negative bacteria are less resistant to HHP than gram-positive bacteria, and so are spores [34]; this has been explained as a result of the complexity of gram-negative cell membranes. The present results agree with this gram-negative susceptibility to HHP treatment, so that an important microbial inactivation was attained.

Table 1. *Shewanella* spp. and *Pseudomonas* spp. assessment (log CFU/g muscle)§ in chilled salmon that was previously treated under different HHP conditions.

Chilled storage time [days]	*Shewanella* spp.				*Pseudomonas* spp.			
	C	T-1	T-2	T-3	C	T-1	T-2	T-3
0	[a]1.96[y]	[a]0.00[z]	[a]0.00[z]	[a]0.00[z]	[a]1.30[y]	[a]0.00[z]	[a]0.00[z]	[a]0.00[z]
	(0.16)	(0.00)	(0.00)	(0.00)	(0.13)	(0.00)	(0.00)	(0.00)
6	[b]3.15	[b]2.66	[b]3.51	[b]3.22	[b]3.46[y]	[b]2.74[z]	[b]2.48[z]	[b]3.58[y]
	(0.16)	(0.39)	(0.69)	(0.09)	(0.06)	(0.43)	(0.06)	(0.29)
10	[c]3.74	[c]3.55	[bc]4.06	[c]4.00	[c]4.94[x]	[c]3.94[zy]	[c]3.99[z]	[c]4.52[yx]
	(0.14)	(0.34)	(0.66)	(0.28)	(0.24)	(0.45)	(0.11)	(0.53)
15	[d]5.31[x]	[d]4.69[y]	[c]4.40[z]	[cd]4.46[z]	[cd]5.24[y]	[d]5.19[zy]	[d]4.81[z]	[cd]5.11[zy]
	(0.09)	(0.03)	(0.08)	(0.18)	(0.06)	(0.22)	(0.16)	(0.14)
20	[e]5.90[x]	[e]4.83[y]	[c]4.57[z]	[d]4.59[z]	[d]5.41	[e]5.58	[e]5.49	[d]5.63
	(0.06)	(0.04)	(0.09)	(0.07)	(0.13)	(0.07)	(0.29)	(0.15)

§ Mean values of three independent determinations ($n = 3$) are given. Standard deviations are indicated in brackets. For each microbial quality index and for each chilling time, mean values followed by different letters (z, y, x) denote significant ($p < 0.05$) differences among treatments. For each microbial quality index and for each treatment, mean values preceded by different letters (a–e) denote significant ($p < 0.05$) differences as a result of chilling time. No letters are included in cases of no significant ($p > 0.05$) differences.
Treatment abbreviations: control (C), 135 MPa for 30 s (T-1), 170 MPa for 30 s (T-2), 200 MPa for 30 s (T-3).

Microbial activity was also measured by chemical indices such as nitrogen from total volatile amines and trimethylamine. With some exceptions, the TVB-N content (Table 2) showed a progressive increase throughout the chilled storage in all kinds of samples, in agreement with the above-mentioned microbial parameters. However, the TVB-N content did not provide a satisfactory correlation with time ($r^2 = 0.67$–0.81, quadratic fitting) or the microbial parameters ($r^2 = 0.55$–0.87, linear fitting) throughout the chilled storage. The TVB-N content found can be considered low when compared to other fish species under similar conditions [1, 29]; however, previous research has already shown a low TVB-N formation for the present species when kept under chilling conditions [16]. No differences ($p > 0.05$) could be assessed among the samples at day 0, so that no effect ($p > 0.05$) of previous HHP treatment could be assessed on the TVB-N content. Then, higher mean values could be observed in control fish individuals than in their corresponding pressure-treated counterparts in the day 15–20 period; however, comparison of the different HHP-treated samples did not provide a clear effect throughout the chilled storage. On the contrary, an inhibition of total volatile amine formation was observed in chilled octopus that was previously pressure treated [10]; in that case, higher pressure and holding time conditions were applied.

For all kinds of samples, the TMA-N content (Table 2) showed important increases ($p < 0.05$) throughout the chilled storage ($r^2 = 0.94$–0.95, logarithmic fitting). These increases were specially marked at day 6, according to values obtained for microbial count parameters (Figs. 1, 2; Table 1). Thus, good correlation values were obtained between the TMA-N content and the microbial parameters throughout the chilled storage for all kinds of samples ($r^2 = 0.91$–0.94, linear fitting). As for the TVB-N values, the contents of TMA-N at day 0

did not provide an inhibitory effect of HHP; for both chemical parameters, it can be argued that the values obtained correspond to those already present in the starting raw fish employed. Later on (days 6–20 of storage), comparison among the different fish samples showed a decreasing TMA-N mean content as long as the pressure previously applied was higher; in most cases, lower ($p < 0.05$) TMA-N values were obtained in fish samples corresponding to T-2 and T-3 conditions when compared to control fish. An inhibitory effect of previous HHP treatment on trimethylamine formation during chilled storage was also found in minced hake muscle [31].

3.2 Lipid analyses related to lipid oxidation development

The lipid content of the employed salmon white muscle was included in the range of 42.5–77.5 g/kg muscle, while the moisture value ranged between 695 and 725 g/kg muscle. These values are in agreement with previous research on this farmed fatty fish species [15, 35].

Primary oxidation (peroxide formation; Fig. 3) showed a progressive formation ($p < 0.05$) throughout the chilled storage time for all kinds of fish samples ($r^2 = 0.89$–0.94, linear fitting). Data obtained at day 0 revealed a low peroxide formation (<3.3 meq active oxygen/kg lipids) and no differences ($p > 0.05$) among samples corresponding to the different treatments, so that no peroxide formation could be concluded after the HHP treatment. On the contrary, previous research [3] showed that the primary oxidation compound (peroxides) content would increase as a result of pressure treatment; however, higher pressures (200–600 MPa) and longer holding times (15–30 min) were employed in such cases when compared to the conditions of the actual research. In the present

Table 2. TVB-N and TMA-N content (mg/kg muscle)[§] in chilled salmon that was previously treated under different HHP conditions.

Chilled storage time [days]	TVB-N				TMA-N			
	C	T-1	T-2	T-3	C	T-1	T-2	T-3
0	[a]289.6 (13.5)	[a]282.8 (29.2)	[a]286.2 (5.8)	[a]289.6 (8.9)	[a]1.3 (0.1)	[a]1.8 (0.0)	[a]1.3 (0.1)	[a]1.6 (0.2)
6	[ab]307.5 (8.5)	[ab]301.4 (1.6)	[ab]307.5 (33.0)	[a]295.2 (8.5)	[b]20.6[x] (0.4)	[b]18.1[y] (0.1)	[b]13.0[z] (1.2)	[b]13.9[z] (0.3)
10	[ab]313.1[y] (10.1)	[a]289.6[z] (12.1)	[a]308.6[zy] (11.8)	[b]319.9[y] (14.7)	[c]24.0[y] (2.3)	[bc]20.6[zy] (3.9)	[c]17.8[z] (1.9)	[c]17.6[z] (2.8)
15	[b]313.5[y] (8.9)	[ab]304.2[zy] (22.4)	[a]291.8[z] (9.7)	[ab]294.1[zy] (15.6)	[d]31.1[y] (2.5)	[c]27.2[zy] (6.2)	[d]22.7[z] (1.5)	[cd]21.6[z] (3.1)
20	[c]352.4[y] (1.9)	[b]335.0[zy] (17.6)	[b]338.4[z] (2.4)	[b]333.2[z] (7.8)	[d]34.0[y] (5.1)	[c]28.8[zy] (3.0)	[e]29.9[zy] (4.7)	[d]24.9[z] (3.7)

[§] Mean values of three independent determinations ($n = 3$) are given. Standard deviations are indicated in brackets. For both quality indices and for each chilling time, mean values followed by different letters (z, y, x) denote significant ($p < 0.05$) differences among treatments. For each quality index and for each treatment, mean values preceded by different letters (a–e) denote significant ($p < 0.05$) differences as a result of chilling time. No letters are included in cases of no significant ($p > 0.05$) differences.
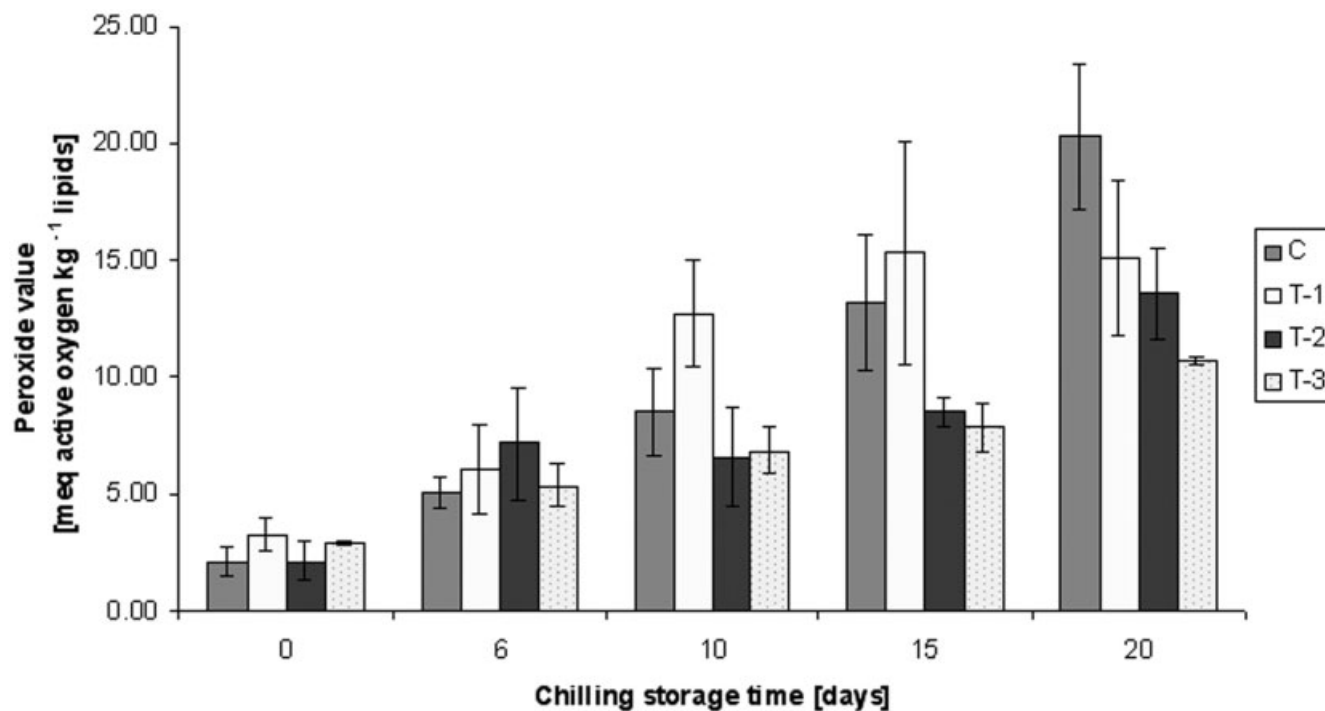Treatment abbreviations as given in Table 1.

Figure 3. Comparative PV assessment in chilled salmon previously treated under different HHP conditions. Mean values of three (n = 3) independent determinations are given. Standard deviations are denoted by bars. Treatment abbreviations as given in Fig. 1.

study, comparison among the samples throughout the chilling time (day 10–20 period) showed lower mean values for individuals corresponding to the T-2 and T-3 treatments than for their counterparts from the two other conditions, with this difference being significant (p <0.05) at day 15.

Secondary lipid oxidation (Table 3) showed a progressive formation (p <0.05) of TBARS throughout the chilled storage for all kinds of samples (r^2 = 0.86–0.90, quadratic fitting). This result is in agreement with the above-mentioned increase of peroxide formation (Fig. 3). The TBARS content at day 0 showed an important effect of pressure treatment so that an increasing TBA-i value was obtained by increasing the applied pressure. Throughout the chilled storage (day 6–15 period), higher (p <0.05) values were obtained for samples corresponding to the highest pressure treatment (T-3) when compared to control fish, so that a pro-oxidant effect of pressure could be concluded in chilled fish. No differences (p >0.05) among the samples could be outlined at the end of the experiment.

An increase of the secondary lipid oxidation compound (TBA-i) content as a result of HHP treatment has also been observed for carpet fillets [36] and turbot fillets [18], both showing an increasing effect with holding time. Previous research also accounts for an increase in TBARS content as a result of HHP treatment followed by a further fish storage/processing; this was the case for chilled rainbow trout [17], cold-smoked salmon [8] and refrigerated (4 °C) cod muscle

[12]. Opposite results were obtained, however, by Ramírez-Suárez and Morrisey [11] when minced albacore muscle was HHP treated and then refrigerated at 4 °C; thus, a lower TBARS formation was found in HHP-treated fish than in control ones.

The results obtained in the present experiment for tertiary lipid oxidation compounds are shown in Table 3. An increasing (p <0.05) formation of fluorescent compounds could be observed for all kinds of samples throughout the day 0–10 period of chilled storage (Table 3). This result is in agreement with the above-mentioned results concerning PV (Fig. 3) and TBA-i (Table 3), and can be explained as a result of chemical reaction between primary and secondary oxidized lipids (namely, electrophilic compounds) and protein-type molecules (namely, nucleophilic compounds) present in the salmon muscle. According to the FR values, no effect (p >0.05) of HHP treatment could be detected at day 0. However, comparison among the different kinds of samples provided differences in the day 10–20 period; thus, control samples and those treated under the lowest pressure conditions (T-1) showed a lower (p <0.05) FR value than their counterpart fish belonging to the T-2 and T-3 treatments.

Lipid oxidation development is recognized as a complex process where different kinds of molecules are produced, most of them unstable and susceptible to breakdown, leading to lower-molecular-weight compounds or reacting with other molecules (mostly of nucleophilic type) present in the fish

Table 3. TBA-i and fluorescence ratio assessment[§] in chilled salmon that was previously treated under different HHP conditions.

Chilled storage time [days]	TBA-i [mg malondialdehyde/kg muscle]				Fluorescence ratio			
	C	T-1	T-2	T-3	C	T-1	T-2	T-3
0	$^a0.35^z$	$^a0.40^{zy}$	$^a0.47^{zy}$	$^a0.61^y$	$^a0.22$	$^a0.23$	$^a0.19$	$^a0.22$
	(0.09)	(0.07)	(0.17)	(0.15)	(0.01)	(0.06)	(0.05)	(0.08)
6	$^b0.58^z$	$^b0.62^{zy}$	$^a0.56^{zy}$	$^{ab}0.83^y$	$^{bc}0.39$	$^{ab}0.33$	$^a0.27$	$^a0.34$
	(0.06)	(0.13)	(0.10)	(0.19)	(0.09)	(0.09)	(0.06)	(0.06)
10	$^b0.50^z$	$^b0.77^y$	$^a0.62^{zy}$	$^{ab}0.79^y$	$^c0.51^z$	$^b0.41^z$	$^b0.64^y$	$^b0.60^y$
	(0.02)	(0.10)	(0.09)	(0.16)	(0.04)	(0.06)	(0.05)	(0.04)
15	$^b0.71^z$	$^b0.78^{zy}$	$^a0.63^z$	$^b0.95^y$	$^{bc}0.45^z$	$^b0.41^z$	$^b0.67^y$	$^b0.68^y$
	(0.21)	(0.28)	(0.18)	(0.12)	(0.03)	(0.08)	(0.13)	(0.06)
20	$^c1.63$	$^c1.61$	$^b1.58$	$^c1.62$	$^b0.40^z$	$^b0.42^z$	$^b0.67^y$	$^b0.64^y$
	(0.10)	(0.12)	(0.03)	(0.15)	(0.03)	(0.07)	(0.11)	(0.02)

[§] Mean values of three independent determinations ($n = 3$) are given. Standard deviations are indicated in brackets. For each chemical quality index and for each chilling time, mean values followed by different letters (z, y) denote significant ($p < 0.05$) differences among treatments. For each quality index and for each treatment, mean values preceded by different letters (a–c) denote significant ($p < 0.05$) differences as a result of chilling time. No letters are included in cases of no significant ($p > 0.05$) differences.
Treatment abbreviations as given in Table 1.

muscle. This would be the case for peroxides, which have been widely reported to breakdown and give rise to secondary and tertiary lipid oxidation compounds [29, 37–39]. The present results concerning lower peroxide contents for fish samples corresponding to T-2 and T-3 conditions agree with this behavior, so that higher TBA-i and FR values were attained for such chilled samples and, accordingly, a higher lipid oxidation development could be inferred. This conclusion also agrees with a reported damage that HHP can induce in protein structure [9, 11], leading to an increasing reactivity of proteins towards electrophilic compound formation [37–39].

HHP treatment has been reported to increase fish muscle lipid oxidation during further storage/processing [3, 12]. However, isolated extracted lipids have been shown to be relatively stable against oxidation under HHP conditions and during further storage. Additionally, the pro-oxidant effect of HHP treatment on muscle lipids was shown to be eliminated if a previous water washing of the muscle was applied or if a complexation compound (EDTA, for example) was added [3, 12]. Consequently, iron-bound protein denaturation during HHP treatment has been reported to facilitate an increase in the free metal ion content and to be responsible for this lipid oxidation development in fish meat after HHP treatment. Since no previous washing was applied to the salmon muscle in the present study, this explanation would agree with the lipid oxidation development found in the actual research.

The PI (Fig. 4) and the tocopherol isomer content (Table 4) were analyzed. Both kinds of parameters did not provide differences ($p > 0.05$) as a result of the previous HHP treatment; this conclusion could be observed at day 0 and later

on (day 6–20 period) during the chilled storage. A PI ranging between 1.50 and 1.65 was found in all cases and can be considered similar to that previously reported for the same farmed species [15, 35].

Concerning tocopherol isomers, three of them (α-, γ- and δ-tocopherol) were detected in the different salmon samples. However, the δ-isomer was found in very low concentrations (<1.0 mg/kg muscle) and could not be quantified satisfactorily. Values obtained for the α-isomer were included in the range of 3.2–7.2 mg/kg muscle, being lower than the values found in previous research concerning the same farmed species [35]. In the case of γ-tocopherol, the values obtained (2.6–4.0 mg/kg muscle) agree with previous results [35]. Large fish-to-fish variations in tocopherol contents were found, especially in the case of the α-isomer.

Previous research has shown an important effect of lipid oxidation development on the PUFA content loss (PI decrease) [29] and on the endogenous antioxidant (tocopherol isomers) content decrease [40] during chilled storage. However, in the present study, in spite of the increasing values found throughout the chilling time for the different lipid oxidation indices (primary, secondary and tertiary), no effect on polyenes and tocopherol isomers could be outlined.

4 Conclusions

The present research provides a valuable employment of HHP technology in order to partially inhibit microbial development throughout a further chilled storage of coho salmon. Since the pressure-holding time conditions employed were chosen as corresponding to the best visual analysis (color,
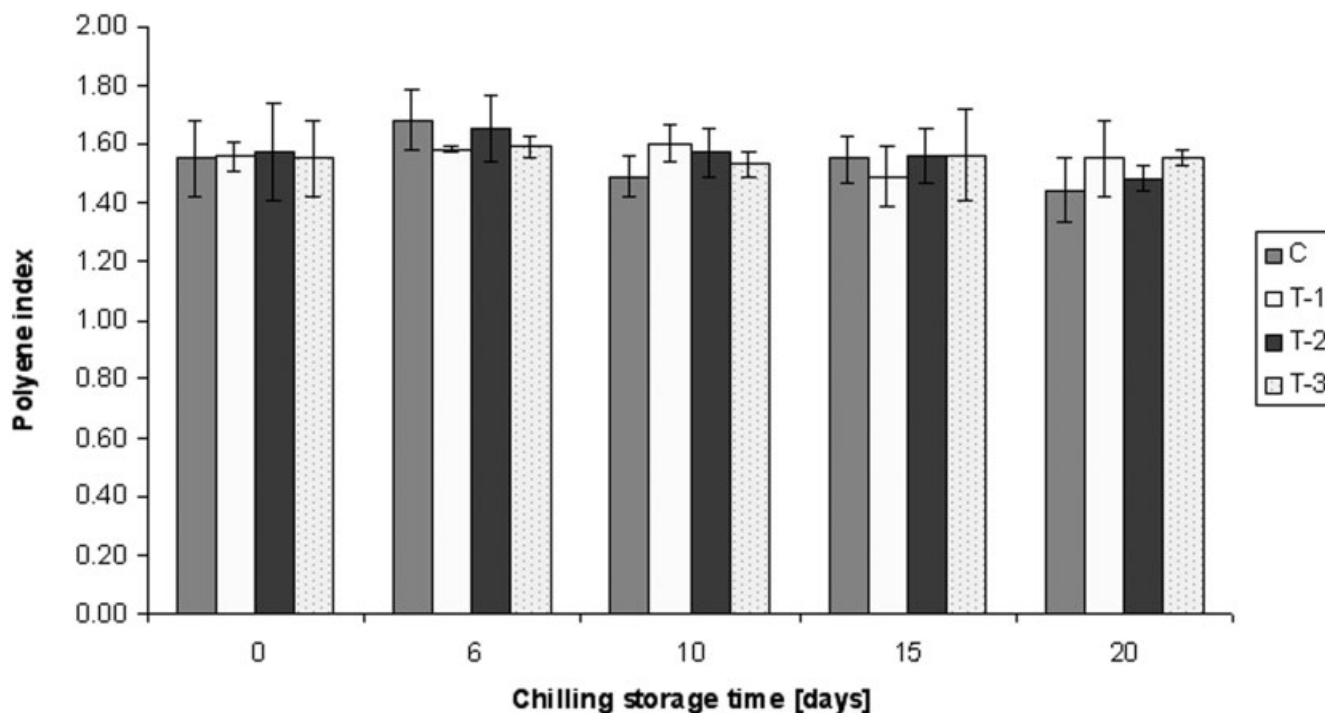
Figure 4. Comparative PI assessment in chilled salmon previously treated under different HHP conditions. Mean values of three (*n* = 3) independent determinations are given. Standard deviations are denoted by bars. Treatment abbreviations as given in Fig. 1.

Table 4. α- and γ-tocopherol content (mg/kg muscle)§ in chilled salmon that was previously treated under different HHP conditions.

Chilling time [days]	α-Tocopherol				γ-Tocopherol			
	C	T-1	T-2	T-3	C	T-1	T-2	T-3
0	[a]5.57 (0.83)	[ab]6.15 (1.20)	[ab]5.92 (1.05)	7.05 (1.52)	[a]2.57 (0.24)	[a]2.58 (0.40)	[a]2.70 (0.32)	2.83 (0.32)
6	[b]7.65 (0.57)	[b]7.62 (0.92)	[b]7.20 (0.77)	6.58 (1.21)	[b]3.72 (0.18)	[b]3.59 (0.34)	[b]4.03 (0.52)	3.30 (0.54)
10	[a]3.94 (1.95)	[a]5.22 (0.97)	[ab]4.99 (2.30)	5.62 (0.40)	[a]2.71 (0.48)	[ab]3.22 (0.97)	[a]2.63 (0.34)	2.58 (0.50)
15	[a]5.24 (1.12)	[ab]6.25 (1.75)	[a]5.23 (1.12)	4.80 (1.25)	[a]3.02 (0.34)	[b]3.72 (0.40)	[ab]3.55 (0.67)	3.24 (0.24)
20	[a]5.14 (1.75)	[ab]6.22 (0.98)	[a]5.13 (0.90)	4.76 (1.47)	[ab]3.19 (0.66)	[ab]3.16 (0.55)	[ab]3.32 (0.79)	2.89 (0.37)

§ Mean values of three independent determinations (*n* = 3) are given. Standard deviations are indicated in brackets. For each tocopherol isomer and for each treatment, mean values preceded by different letters (a, b) denote significant (*p* <0.05) differences as a result of chilling time; no letters are included in cases of no significant (*p* >0.05) differences. No significant differences (*p* >0.05) were obtained as a result of previous high-pressure treatment.
Treatment abbreviations as given in Table 1.

gaping, elasticity and firmness) scores found, a good agreement can be denoted between sensory acceptance and microorganism inhibition for this farmed species.

Examination of previous research shows that lipid oxidation analysis has been scarce when compared to the number of studies focusing on the microbial activity and the protein

deteriorative changes as a result of HHP technology employment in fish products; in addition, a single lipid oxidation index has been checked in most cases.

In the present study, the lipid oxidation progress was evaluated at different stages, so that a wide knowledge of the effect of HHP treatment on the lipid oxidation mechanism

could be attained. Thus, the assessment of primary, secondary and tertiary lipid oxidation compounds has shown a lipid oxidation development in all kinds of samples by increasing the chilling time. However, a greater lipid oxidation development could be outlined in chilled fish corresponding to the two highest pressure conditions (T-2 and T-3), since higher secondary (TBA-i) and tertiary compound (FR) values could be observed when compared to their counterpart fish corresponding to control and T-1 conditions. Meanwhile, the primary oxidation compound (PV) content was found lower for T-2 and T-3 fish, according to a reported breakdown process that may favor the secondary and tertiary lipid oxidation compound formation. In spite of this lipid oxidation development, no effect on the PI and on the tocopherol isomer content was attained. Accordingly, it can be considered that T-2 and T-3 HHP conditions would contribute to the lipid nutritional value preservation in addition to the quality and safety loss inhibition during chilled storage of farmed coho salmon.

Acknowledgments

Conflict of interest statement

The authors have declared no conflict of interest.

References

[1] K. Whittle, R. Hardy, G Hobbs: Chilled fish and fishery products. In: *Chilled Foods. The State of the Art*. Ed. T. Gormley, Elsevier Applied Science, New York, NY (USA) 1990, pp 87–116.

[2] G. Olafsdóttir, E. Martinsdóttir, J. Oehlenschläger, P. Dalgaard, B. Jensen, I. Undeland, I. Mackie, G. Henehan, J. Nielsen, H. Nilsen: Methods to evaluate fish freshness in research and industry. *Trends Food Sci Technol*. 1997, **8**, 258–265.

[3] T. Ohshima, H. Ushio, C. Koizumi: High-pressure processing of fish and fish products. *Trends Food Sci Technol*. 1993, **4**, 370–375.

[4] L. Murchie, M. Cruz-Romero, J. Kerry, M. Linton, M. Patterson, M. Smiddy, A. Kelly: High pressure processing of shellfish: A review of microbiological and other quality aspects. *Innov Food Sci Emerg Technol*. 2005, **6**, 257–270.

[5] P. Montero, M. Pérez-Mateos, A. J. Borderías: Chilled storage of high pressure and heat-induced gels of blue whiting (*Micromesistius poutassou*) muscle. *Z Lebensm Unters Forsch*. 1998, **207**, 146–153.

[6] G. Tabilo-Munizaga, G. Barbosa-Cánovas: Ultra high pressure technology and its use in surimi manufacture: An overview. *Food Sci Technol Int*. 2004, **10**, 207–222.

[7] J. Rouillé, A. Le Bail, H. Ramaswamy, L. Leclerc: High pressure thawing of fish and shellfish. *J Food Eng*. 2002, **53**, 83–88.

[8] R. Lakshmanan, J. Parkinson, J. Piggott: High-pressure processing and water-holding capacity of fresh and cold-smoked salmon (*Salmo salar*). *Food Sci Technol*. 2007, **40**, 544–551.

[9] I. Ashie, B. Simpson: Application of high hydrostatic pressure to control enzyme related fresh seafood texture deterioration. *Food Res Int*. 1996, **29**, 569–575.

[10] J. Hurtado, P. Montero, A. J. Borderías: Chilled storage of pressurized octopus (*Octopus vulgaris*) muscle. *J Food Sci*. 2001, **66**, 400–406.

[11] J. Ramírez-Suárez, M. Morrisey: Effect of high pressure processing (HPP) on shelf life of albacore tuna (*Thunnus alalunga*) minced muscle. *Innov Food Sci Emerg Technol*. 2006, **7**, 19–27.

[12] K. Angsupanich, D. Ledward: High pressure treatment effects on cod (*Gadus morhua*) muscle. *Food Chem*. 1988, **63**, 39–50.

[13] FAO: *Fishery Statistics. Aquaculture Production*. Food and Agriculture Organization of the United Nations, Rome (Italy) 2007, Yearbook 2005, 100/2, 73.

[14] FAO: *Fishery Statistics. Capture Production*. Food and Agriculture Organization of the United Nations, Rome (Italy) 2007, Yearbook 2005, 100/1, 79.

[15] S. Aubourg, J. Vinagre, A. Rodríguez, V. Losada, Mª A. Larraín, V. Quitral, J. Gómez, L. Maier, E. Wittig: Rancidity development during the chilled storage of farmed coho salmon (*Oncorhynchus kisutch*). *Eur J Lipid Sci Technol*. 2005, **107**, 411–417.

[16] S. Aubourg, V. Quitral, Mª A. Larraín, A. Rodríguez, J. Gómez, L. Maier, J. Vinagre: Autolytic degradation and microbiological activity in farmed coho salmon (*Oncorhynchus kisutch*) during chilled storage. *Food Chem*. 2007, **104**, 369–375.

[17] Y. Yagiz, H. Kristinsson, M. Balaban, M. Marshall: Effect of high pressure treatment on the quality of rainbow trout (*Oncorhynchus mykiss*) and mahi mahi (*Coryphaena hippurus*). *J Food Sci*. 2007, **72**, C509–C515.

[18] D. Chevalier, A. Le Bail, M. Ghoul: Effects of high pressure treatment (100–200 MPa) at low temperature on turbot (*Scophthalmus maximus*) muscle. *Food Res Int*. 2001, **34**, 425–429.

[19] K. Van Spreekens: The suitability of a modification of Long and Hammer's medium for the enumeration of more fastidious bacteria from fresh fishery products. *Arch Lebensmittelhyg*. 1974, **25**, 213–219.

[20] N. Kovacs: Identification of *Pseudomonas pyocyanae* by the oxidase reaction. *Nature* 1956, **178**, 703.

[21] R. Hugh, E. Leifson: The taxonomic significance of fermentative *versus* oxidative gram-negative bacteria. *J Bacteriol*. 1953, **66**, 24–26.

[22] L. Gram, G. Trolle, H. Huss: Detection of specific spoilage bacteria from fish stored at low (0 °C) and high (20 °C) temperatures. *Int J Food Microbiol*. 1987, **4**, 65–72.

[23] S. Aubourg, C. Sotelo, J. Gallardo: Quality assessment of sardines during storage by measurement of fluorescent compounds. *J Food Sci*. 1997, **62**, 295–298, 304.

[24] H. Tozawa, K. Erokibara, K. Amano: Proposed modification of Dyer's method for trimethylamine determination in cod-

fish. In: *Fish Inspection and Quality Control.* Ed. R. Kreuzer, Fishing News Books, London (UK) 1971, pp 187–190.

[25] AOAC: *Official Methods of Analysis of the Association of Analytical Chemistry.* 15th Edn. Ed. K. Helrich, AOAC, Arlington, VA (USA) 1990, p 931.

[26] E. Bligh, W. Dyer: A rapid method of total extraction and purification. *Can J Biochem Physiol.* 1959, **37**, 911–917.

[27] R. Chapman, J. McKay: The estimation of peroxides by the ferric thiocyanate method. *J Am Oil Chem Soc.* 1949, **26**, 360–363.

[28] W. Vyncke: Direct determination of the thiobarbituric acid value in trichloroacetic acid extracts of fish as a measure of oxidative rancidity. *Fette Seifen Anstrichm.* 1970, **72**, 1084–1087.

[29] S. Aubourg, I. Medina, J Gallardo: Quality assessment of blue whiting (*Micromesistius poutassou*) during chilled storage by monitoring lipid damages. *J Agric Food Chem.* 1998, **46**, 3662–3666.

[30] L. Cabrini, L. Landi, C. Stefanelli, V. Barzanti, A. Sechi: Extraction of lipid and lipophilic antioxidants from fish tissues: A comparison among different methods. *Comp Biochem Physiol B Biochem Mol Biol.* 1992, **101**, 383–386.

[31] J. Hurtado, P. Montero, A. J. Borderías: Extension of shelf life of chilled hake (*Merluccius capensis*) by high pressure. *Food Sci Technol Int.* 2000, **6**, 243–249.

[32] M. Pérez-Won, G. Tabilo-Munizaga, G. Barbosa-Cánovas: Effects of ultra high pressure on bay scallop (*Aequipecten irradians*) adductor muscles. *Food Sci Technol Int.* 2005, **11**, 477–484.

[33] R. Chéret, N. Chapleau, C. Delbarre-Ladrat, V. Vérrez-Bagnis, M. De Lamballerie: Effects of high pressure on texture and microstructure of sea bass (*Dicentrarchus labrax* L.) fillets. *J Food Sci.* 2005, **70**, E477–E483.

[34] L. Gram, H. Huss: Flesh and processed fish and shellfish. In: *The Microbiological Safety and Quality of Food. Vol. 1.* Eds. B. Lund, T. Baird-Parker, Aspen Publishers, Frederick, MD (USA) 2000, pp 472–506.

[35] A. Rodríguez, V. Losada, Mª A. Larraín, V. Quitral, J. Vinagre, S. Aubourg: Development of lipid changes related to quality loss during the frozen storage of farmed coho salmon (*Oncorhynchus kisutch*). *J Am Oil Chem Soc.* 2007, **84**, 727–734.

[36] A. Sequeira-Muñoz, D. Chevalier, A. Le Bail, H. Ramaswamy, J. Simpson: Physicochemical changes induced in carp (*Cyprinus carpio*) fillets by high pressure processing at low temperature. *Innov Food Sci Emerg Technol.* 2006, **7**, 13–18.

[37] J. Pokorný: Browning from lipid-protein interactions. *Prog Food Nutr Sci.* 1981, **5**, 421–428.

[38] S. Aubourg: Review: Recent advances in assessment of marine lipid oxidation by using fluorescence. *J Am Oil Chem Soc.* 1999, **76**, 409–419.

[39] V. Tironi, M. Tomás, Mª C. Añón: Structural and functional changes in myofibrillar proteins of sea salmon (*Pseudopercis semifasciata*) by interaction with malondialdehyde (RI). *J Food Sci.* 2002, **67**, 930–935.

[40] I. Undeland, G. Hall, H. Lingnert: Lipid oxidation in fillets of herring (*Clupea harengus*) during ice storage. *J Agric Food Chem.* 1999, **47**, 524–532.