Inhibition of lipid damage in refrigerated salmon (Oncorhynchus kisutch) by a combined treatment of CO$_2$ packaging and high-pressure processing

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The effect of a previous combined treatment (CO₂-enriched modified atmosphere packaging, MAP, and high-pressure processing, HPP, 150 MPa/5 min) on lipid stability of refrigerated (10 days/4 °C) salmon (*Oncorhynchus kisutch*) was studied. The following processing conditions were compared: B-0 (fish without MAP or HPP), B-1 (fish packaged under MAP and without HPP), B-2 (fish subjected to HPP without MAP) and B-3 (fish subjected to MAP and HPP). An inhibitory effect (*p*<0.05) on lipid hydrolysis and oxidation was obtained by the presence of CO₂ in the packaging medium; values detected at day 10 for B-0 and B-1 fish were 80.72 and 49.61 (g free fatty acids, FFA, kg⁻¹ lipids), 6.14 and 2.81 (meq active oxygen·kg⁻¹ lipids; peroxide value), 5.05 and 3.10 (mg malondialdehyde·kg⁻¹ muscle), and 5.56 and 2.70 (fluorescence ratio), respectively. Furthermore, inhibition of lipid damage was observed for HPP alone; values detected at day 10 for B-2 fish were 76.24 (g FFA·kg⁻¹ lipids) and 5.28 (meq active oxygen·kg⁻¹ lipids). The lowest average values for lipid hydrolysis and oxidation were obtained in samples corresponding to the combined treatment (B-3 batch), differences being significant (*p*<0.05) at day 10 for FFA (41.43 g·kg⁻¹ lipids), peroxide (1.84 meq·kg⁻¹ lipids) and fluorescence (2.50) values.

**Keywords**: Coho salmon; modified atmosphere; high-pressure processing; refrigeration; lipid hydrolysis; lipid oxidation; quality enhancement.

**Running head**: Salmon quality and combined CO₂ packaging/HPP.
INTRODUCTION

Modified atmosphere packaging (MAP) is a convenient method widely used to preserve the freshness of marine species and to maintain their nutritional, sanitary and sensory characteristics, so that an increased shelf-life is achieved (Gokoglu, 2020; Kuley et al., 2020). It involves the total or partial removal of atmospheric air contained in the package by reintroduction of other gases, usually CO₂, N₂ and O₂, alone or in combination. Its application to refrigerated seafood at different gas concentrations (%) leads to a marked inhibition of microbial modifications and lipid oxidation development (Masniyom et al., 2002; Sun et al., 2017; Zhou et al., 2020). However, if relatively long storage periods or high storage temperatures are required, seafood quality may be lost. Consequently, the possibilities of using MAP for enhancing the quality and increasing the shelf-life of seafood have been investigated by combining it with other preserving strategies such as superchilled storage (Fagan et al., 2004), UV-C irradiation (Leal Rodrigues et al., 2016), edible preservative coatings (Carrió-Granda et al., 2018) and treatment with preserving compounds such as essential oils (Giatrakou et al., 2008), potassium sorbate (Yesudhason et al., 2010) or pink pepper extract (Cardoso Merlo et al., 2019).

Another preserving technology for fatty and lean marine species is high-pressure processing (HPP). This technology has been proved to inactivate the development of microbes (aerobes, anaerobes, psychrotrophs, Shewanella spp., Pseudomonas spp., etc.) and deteriorative endogenous enzymes (proteases, lipases, phospholipases, lipoxygenases, oxidases, peroxidases, etc.), so that an increase in shelf-life is produced during subsequent refrigerated or frozen storage (Campus, 2010; Alves de Oliveira et al., 2017). However, deteriorative problems have been encountered with constituents like proteins and lipids when relatively high pressure levels are employed (Medina-Meza et al., 2019).
al., 2014; Huang et al., 2017; Aubourg, 2018). In order to avoid such chemical
modifications, the combination of HPP with other preserving strategies has been checked,
the effects being analysed during subsequent refrigerated storage. Among such
complementary strategies, the employment of functional edible films (Ojagh et al., 2011),
short thermal treatment (75 °C for 5 min) (Chouhan et al., 2015) and the addition of
preservative compounds (Pérez-Mateos et al., 2002) have been tested satisfactorily.

Previous research has studied the employment of combined MAP and HPP on
seafood. Such combination would provide the advantages of each of the processes if
considered alone and may also lower the pressure requirement. Thus, a marked
inactivation of microbial activity and an increase in shelf-life was observed in refrigerated
(5 °C) Atlantic salmon (Salmo salar) subjected to MAP (50/50, CO₂/O₂) and HPP (150
MPa for 15 min) (Amanatidou et al., 2000). Later on, Jing et al. (2015) showed that HPP
(290 MPa for 6 min) combined with MAP (50-60/0-15/25-40, CO₂/O₂/N₂) was effective
on inhibiting total bacteria count and total volatile base nitrogen during the cold storage
(21 days at 4 °C) of cutlassfish (Trichiurus lepturus). Recently, a preserving effect by
inactivating endogenous enzymes (i.e., proteases and lipases) as well as aerobe and
psychrophilic microorganism activity was detected in refrigerated (4 °C) salmon
(Oncorhynchus kisutch) subjected to MAP (50-100% CO₂) and HPP (150 MPa for 5 min)
(Pérez-Won et al., 2020).

Among farmed fish, Coho salmon (Oncorhynchus kisutch) is consumed
worldwide, leading to a continuous increase in production. Previous research related to
the chilled storage of this species has investigated the development of different damage
mechanisms and quality losses (Ortea et al., 2010; Rodríguez et al., 2016), lipid damage
being found to be especially important according to the species’ high total lipid and PUFA
content (Vinagre et al., 2011). Consequently, the present study focused on the
development of lipid damage produced during the refrigerated storage of this cultivated species. In it, a combination of MAP and HPP was applied to enhance the lipid quality. On the basis of the results obtained in the above-mentioned study (Pérez-Won et al., 2020), the effect of CO₂-enriched MAP and HPP (150 MPa for 5 min) on the development of lipid hydrolysis and oxidation at different stages in refrigerated Coho salmon muscle was analysed. The working hypothesis was that development of lipid damage can be minimised by inhibiting endogenous enzyme activity (HPP action) as well as by minimising the presence of O₂ in the packaging medium (CO₂-enriched MAP action).

MATERIAL AND METHODS

Raw material, processing and sampling

Coho salmon (Oncorhynchus kisutch) was obtained from Salones Aysen S. A. (Puerto Montt, Chile), where fish samples were beheaded, gutted and filleted in a refrigerated room (4 °C); according to visual examination, pieces including a mechanical damage were discarded. Once at the laboratory, 78 fillets (165 g each) without mechanical damage and a thickness of approximately of 10.5 ± 0.2 mm were selected for the study. Six fillets were taken and divided into three groups (two fillets per group), the white muscle being separated, pooled together within each group, minced and analysed independently (n = 3) to assess the raw fish quality. The remaining 72 fillets were individually packaged in low-density polyethylene bags (15 × 18 cm, 0.050 mm thickness; water vapour transmission rate lower than 20 g/m²·d measured at 40 °C and 90% relative humidity; oxygen permeability lower than 10 cm³/m²·d·atm) and divided into four batches (B-0, B-1, B-2 and B-3) (18 fillets per batch) subjected to the following processes: B-0 (fish without MAP and not subjected to HPP; control batch), B-1 (fish subjected to MAP and not subjected to HPP), B-2 (fish subjected to HPP without MAP) and B-3 (fish subjected to both MAP and HPP).
Accordingly, bags corresponding to batches B-0 and B-2 were vacuum-sealed in a double chamber machine DuoMAT 450 (Webomatic, Bochum, Germany). On the other side, the air inside bags corresponding to batches B-1 and B-3 was removed and flushed with food-grade CO₂ (Indura, Santiago, Chile) for 20 s to ensure a CO₂-enriched atmosphere and immediately sealed without gas loss (head space ca. 20%). Then, bags corresponding to batches B-2 and B-3 were subjected to HPP. For that, an isostatic pressing system (Avure Inc., Kent, WA, USA) with a cylindrical pressure chamber (length: 700 mm; diameter: 60 mm) was employed, water being used as the pressurising medium. A 150 MPa pressure level with a 5 min holding time was applied at a rate of 17 MPa·s⁻¹ at room temperature (20.0 ± 2.0 °C).

Bags corresponding to all batches were stored in a refrigerated room (4 °C) in the dark. Salmon samples were taken for analysis on days 3, 7 and 10 of storage. At each sampling point and for each batch condition, six fillets were taken and divided into three groups (two fillets per group), the white muscle being separated, pooled together within each group, minced and analysed independently (n = 3).

**Extraction of lipid fraction**

Lipids from the salmon white muscle were extracted following the method of Bligh and Dyer (1959) in which single-phase solubilisation of the lipids was employed by means of a chloroform–methanol (1:1) mixture. Results were calculated as g lipid·kg⁻¹ salmon muscle.

**Free fatty acid determination**

The free fatty acid (FFA) content was determined using the lipid extract of the fish white muscle according to the method developed by Lowry and Tinsley (1976).
method is based on the formation of a complex between FFA and cupric acetate–pyridine, followed by spectrophotometric determination at 715 nm (Beckman Coulter DU 640 spectrophotometer, Brea, CA, USA). Results were calculated as g FFA·kg$^{-1}$ lipids.

**Assessment of lipid oxidation**

Conjugated diene (CD) formation was measured at 233 nm (Kim & Labella, 1987) and calculated as $CD = \frac{A \cdot V \cdot w}{1}$, where $A$ is the absorbance reading and $V$ and $w$ are the volume (mL) and weight (mg), respectively, of the lipid extract aliquot taken for analysis.

The peroxide value (PV) was determined spectrophotometrically (520 nm) according to the method developed by Chapman and McKay (1949) in which peroxides included in the lipid extract are reduced with ferric thiocyanate. Results were calculated as meq. active oxygen·kg$^{-1}$ lipids.

The thiobarbituric acid index (TBA-i) was determined according to the method proposed by Vyncke (1970). This method is based on the reaction between a trichloroacetic acid extract of the salmon white muscle and thiobarbituric acid. In it, the content of thiobarbituric acid reactive substances (TBARS) is spectrophotometrically measured at 532 nm. For quantitative purposes, a standard curve using 1,1,3,3-tetraethoxy-propane (TEP) was employed. Results were calculated as mg malondialdehyde·kg$^{-1}$ muscle.

The formation of fluorescent compounds (Fluorimeter LS 45; Perkin Elmer España; Tres Cantos, Madrid, Spain) was determined in the lipid extract of the salmon white muscle by measurements at 393/463 nm and 327/415 nm as described by Aubourg *et al.* (2020). The relative fluorescence (RF) was calculated as follows: $RF = \frac{F}{F_{st}}$, where $F$ is the fluorescence measured at each excitation/emission wavelength pair and $F_{st}$ is the fluorescence intensity of a quinine sulphate solution (1 µg·mL$^{-1}$ in 0.05 M H$_2$SO$_4$) at the
corresponding wavelength pair. Results are given as the fluorescence ratio (FR), which was calculated as the ratio between the two RF values: \( FR = \frac{RF_{393/463 \text{ nm}}}{RF_{327/415 \text{ nm}}} \).

**Statistical analysis**

Experiments were carried out in triplicate \((n = 3)\). Data corresponding to lipid damage (hydrolysis and oxidation indices) were subjected to ANOVA to explore differences resulting from the effect of MAP, HPP and refrigeration time. In all cases, analyses were carried out using PASW Statistics 18 software for Windows (SPSS Inc., Chicago, IL, USA). The comparison of means was performed using the least-squares difference (LSD) method. Differences among batches were considered significant for a confidence interval at the 95% level \((p < 0.05)\).

**RESULTS AND DISCUSSION**

**Determination of lipid hydrolysis**

An increase of the FFA content was observed in all batches with the refrigeration time (Table 1); this increase was found particularly high \((p < 0.05)\) in fish corresponding to batches without MAP. Consequently, a marked inhibitory effect on the development of lipid hydrolysis was proved as a result of subjecting this fatty fish species (lipid content: 95.0–125.0 g·kg\(^{-1}\) muscle) to CO\(_2\)-atmosphere packaging. Furthermore, lower average values were obtained in fish corresponding to the batch subjected only to HPP \((i.e., \text{batch B-2})\) than in the control one; differences were significant \((p < 0.05)\) after 7 days of storage. Therefore, it is concluded that HPP prior to refrigerated storage has led to an inhibitory effect on FFA formation. Interestingly, salmon muscle corresponding to batch B-3 (namely, combined MAP and HPP) provided the lowest average values
throughout the whole storage period, differences being significant ($p < 0.05$) for the 7–10-day period when compared with fish corresponding to any other batch.

FFA formation in refrigerated fish muscle has been reported to be a result of endogenous enzyme activity and microbial development (Sikorski & Kolakowski, 2000; Campos et al., 2012). Before the end of the microbial lag phase, endogenous enzyme activity should be more important; after that time, microbial activity should gain importance and be mostly responsible for the development of lipid hydrolysis. According to the results obtained, both HPP and MAP have shown an inhibitory effect on the development of lipid hydrolysis. Notably, since fish corresponding to batch B-3 showed the lowest formation of FFA, it is concluded that the combination of both preservative processes is useful to inhibit the development of lipid hydrolysis. To the best of our knowledge, no previous research accounts for the detection of lipid hydrolysis in refrigerated fish after being subjected to MAP/HPP combination.

The inhibitory effect on lipid hydrolysis of the current processing combination can be explained on the basis of the effects of both single treatments. For HPP, the preservative effect can be explained by the denaturation of lipolytic enzymes (phospholipases and lipases in general; endogenous and microbial) and, consequently, inhibition of their activity during subsequent refrigerated storage (Medina-Meza et al., 2014; Alves de Oliveira et al., 2017). Interestingly, FFA formation would not be likely to occur as a result of direct high-pressure treatment since the resulting volume increase would not be favoured by the Le Châtelier rule (Campus, 2010). The fact that significant differences ($p < 0.05$) were obtained scarcely by HPP alone can be explained on the basis of employing a relatively mild high-pressure level (Aubourg, 2018).

In the case of MAP, the preservative effect can be explained as a result of the strong antibacterial behaviour reported for MAP in general, and especially for a CO$_2$-
enriched atmosphere. Such a MAP condition has shown a great ability to provide an acidic
medium on the muscle surface (i.e., the formation of carbonic acid), which in turn would
substantially inactivate microbial enzymes (Kuley et al., 2020). This preservative effect
was detected in the current study when taking into account advanced storage times (7–10
days).

Previous research has reported on the effect of HPP on the development of lipid
hydrolysis during subsequent refrigerated storage of fish. In agreement with the current
results, Ortea et al. (2010) concluded a pressure-dependent effect on FFA formation
during subsequent chilled storage of Coho salmon (Oncorhynchus kisutch). Thus, the best
results were obtained when applying the highest pressure tested (i.e., 200 MPa for 30 s)
in samples stored for a 15–20-day period. Later on, Maluenda et al. (2013) subjected
Chilean jack mackerel (Trachurus murphyi) to HPP (250–550 MPa for 3–4 min) followed
by 14-day storage in ice. As a result, an inhibitory effect on FFA formation throughout
chilled storage was observed with increasing pressure level and pressure-holding time.

Furthermore, the effect of HPP (250 and 350 MPa for 10 min) on the formation of FFA
in hilsa (Tenualosa ilisha) fillets was investigated during a 25-day storage period at 4 °C
(Chouhan et al., 2015). The study revealed an inhibitory effect of high pressure on FFA
formation during the storage period, the effect increasing with the pressure level applied.

Previous research related to the effect of MAP on FFA formation in seafood can
be considered scarce. Thus, Fagan et al. (2004) analysed FFA formation in freeze-chilled
mackerel and salmon packaged under different MAP conditions. As a result, 100% CO2
MAP led to the highest FFA values in stored mackerel. On the contrary, this packaging
condition led to the lowest FFA values when applied to salmon storage.
Determination of lipid oxidation

Lipid oxidation was followed by assessing different kinds of compounds produced during the development of this damage mechanism. The formation of CD and peroxides (primary compounds), TBARS (secondary compounds) and fluorescent compounds (tertiary compounds) was taken into account and assessed throughout the refrigerated storage.

A slight increasing tendency of CD values with refrigeration time could be observed in all batches (Table 2). This increase was more important in fish corresponding to batches not including MAP; differences were significant ($p < 0.05$) at the end of the storage period. Additionally, comparison between fish corresponding to batches B-0 and B-2 showed the lowest average values in previously pressurised fish although significant differences were not obtained ($p > 0.05$). Notably, the combination of MAP and HPP under the present conditions led to the lowest average values at all refrigeration times although significant differences ($p > 0.05$) were not observed when compared with fish corresponding to the MAP batch (B-1).

Low formation of peroxides was detected in all cases during the 3–7-day period. Remarkably, substantial formation of peroxides was observed at the end of the experiment in salmon muscle corresponding to the control and batch B-2 (Table 3); however, values did not exceed a PV of 6.2. Therefore, it is considered that the formation of this kind of oxidation products was not relevant in the current experiment. Comparison between fish corresponding to batches B-0 and B-1 revealed an inhibitory effect on peroxide formation for the MAP treatment, differences being significant for the 7–10-day period. Related to the HPP effect, lower average values were detected in fish corresponding to batch B-2 than in their counterparts from the control batch; differences were only significant ($p < 0.05$) at the end of the experiment. Interestingly, the lowest
average values were obtained in salmon muscle corresponding to the combined preserving condition (i.e., batch B-3) for the 7–10-day period; compared with all other batches under study, differences were significant ($p < 0.05$) at the end of the experiment.

A substantial increase in TBARS content was detected in all cases throughout the storage period (Table 4). As for primary oxidation compounds (namely, CD and peroxides), a smaller increase of TBARS content was found ($p < 0.05$) in fish subjected to MAP than in batches B-0 and B-2. As a result, the assessment of this kind of oxidation molecules revealed the lowest values ($p < 0.05$) in fish corresponding to batches B-1 and B-3 throughout the whole storage period. Additionally, the combination of MAP and HPP led to lower average values than for any other treatment condition. Remarkably, differences were significant ($p < 0.05$) when compared with fish corresponding to batches B-0 and B-2 but not ($p > 0.05$) with fish corresponding to MAP alone.

Formation of interaction compounds between oxidised lipids (primary and secondary) and nucleophilic compounds present in the salmon muscle (mostly protein-type molecules) was measured by assessment of the formation of fluorescent compounds (Table 5). A general progressive increase ($p < 0.05$) with refrigeration time was detected, this increase being higher ($p < 0.05$) in samples corresponding to batches B-0 and B-2. Consequently, a strong inhibitory effect was concluded for the MAP condition tested in the current study; notably, differences from the control batch were significant ($p < 0.05$) throughout the whole storage period. Concerning the HPP effect, lower average values were detected in B-2 fish than in their counterpart control; however, differences were not significant ($p > 0.05$). Interestingly, the lowest average values were obtained during the whole storage period in the fish batch corresponding to the combined strategy (B-3 batch); differences were significant ($p < 0.05$) for extended storage times.
According to the current results obtained for the different lipid oxidation indices, an inhibitory effect on lipid oxidation can be signalled for the conditions tested for MAP and HPP when considered alone. This effect showed to be increased for the MAP and HPP combination as a result of the effects exerted by each of the processes applied. Furthermore, chemical indices corresponding to all steps (primary, secondary, and tertiary) of lipid oxidation development have shown to be accurate tools for assessing quality changes.

Concerning HPP, protein denaturation can lead to two opposite effects on the development of lipid oxidation. On one side, the high-pressure denaturation of transition metal-binding proteins can facilitate an increase of free metal ion content, this leading to an increased catalysation effect on lipid oxidation during the subsequent refrigerated storage. On the other side, the denaturation of pro-oxidant endogenous enzymes (lipoxygenases, oxidases, peroxidases, etc.) during HPP can lead to inhibition of lipid oxidation during the subsequent refrigerated storage (Medina-Meza et al., 2014; Aubourg, 2018). Comparison between the current results obtained from batches B-0 and B-2 reveals that the inhibitory effect of HPP (namely peroxide and TBARS values) was slightly higher than the pro-oxidant one, leading to an additional quality enhancement in fish corresponding to batch B-3 as a result of combining it with the MAP treatment. The fact that significant differences ($p < 0.05$) were obtained scarcely by HPP alone can be explained on the basis of employing a relatively mild high-pressure level (Aubourg, 2018).

On the other side, MAP led to a marked antioxidant effect, according to the comparison of results obtained in fish corresponding to batches B-0 and B-1. This effect can be explained as a result of employing a $\text{CO}_2$-enriched atmosphere. Thus, $\text{O}_2$ presence in the packaging medium would be negligible and consequently, lipid rancidity would be
minimised during the subsequent refrigerated storage of the present fatty fish species. However, total inhibition of the development of lipid oxidation was not produced in fish corresponding to batches B-1 and B-3. As an explanation, a residual presence of O$_2$ in packaged fish muscle is expected to occur that can facilitate lipid oxidation development. Furthermore, the packaging material employed may allow minimal gas transmission (i.e., O$_2$ and CO$_2$), so that there may be an increase in O$_2$ content inside the packaging medium with storage time, favouring the development of lipid oxidation. In the case of B-2 and B-3 fish, this effect could be more important on the basis that previous HPP has been reported to increase the permeability of most packaging materials throughout subsequent storage (Bull et al., 2010; Marangoni Júnior et al., 2020).

Previous research concerning the effect of combined MAP and HPP on lipid oxidation development in fish has provided contradictory results. Thus, Amanatidou et al. (2000) detected a detrimental effect on oxidative rancidity (TBARS assessment) in refrigerated salmon (S. salar) muscle that could not be proved to be exclusively due to the gas mixture present in the atmosphere (50/50, CO$_2$/O$_2$) and HPP (150 MPa for 10 min). Combination of HPP treatment (290 MPa for 6 min) with MAP (50-60/0-15/25-40, CO$_2$, O$_2$, and N$_2$, respectively) led to an increased TBARS formation by increasing the presence of O$_2$ in the packaging system in refrigerated cutlassfish (T. lepturus) (Jing et al., 2015); however, fish corresponding to all treated batches provided lower TBARS values than fish corresponding to open-air condition. Recently, Pérez-Won et al. (2020) observed a higher oxidation level (TBARS assessment) in refrigerated salmon (O. kisutch) previously submitted to MAP (50%-CO$_2$) and HPP (150 MPa for 5 min) after a 10-day storage.

A wide range of previous studies have investigated the effect of HPP on the development of lipid oxidation in refrigerated seafood. Remarkably, such studies lead to
opposite conclusions and have been carried out on a single lipid oxidation index (namely, TBARS value). In agreement with the present results, HPP was revealed to have an inhibitory effect on TBARS formation in albacore (*Thunnus alalunga*) muscle (Ramírez-Suárez & Morrisey, 2006), red mullet (*Mullus surmuletus*) muscle (Erkan *et al*., 2010), and hilsa (*Tenualosa ilisha*) fillets (Chouhan *et al*., 2015) throughout subsequent refrigerated storage. Contrary to such results, increased TBA values were revealed in rainbow trout (*Oncorhynchus mykiss*) (Yagiz *et al*., 2007), and cod (*Gadus morhua*) and salmon (*Salmo salar*) (Rode & Hovda, 2016) during refrigerated storage as a result of previous HPP. Other studies have shown a different effect according to the pressure level applied. Thus, Lakshmanan *et al*. (2005) found that pressure levels not higher than 200 MPa could be optimal for enhancement of the lipid quality (TBA value assessment) of vacuum-packaged cold-smoked salmon (*Salmo salar*). Furthermore, substantial lipid oxidation (TBA and fluorescence indices) was observed in chilled Coho salmon (*Oncorhynchus kisutch*) if previously subjected to 200 MPa for 30 s (Aubourg *et al*., 2010); however, increased lipid stability was proved if treatment of 135 MPa for 30 s was previously applied.

Concerning the effect of CO2-enriched MAP on the development of lipid oxidation, previous research accounts for different conclusions, according to the experimental conditions concerned. Thus, a lower TBARS formation in vacuum-packaged and refrigerated Chub mackerel was observed by Erkan *et al*. (2007) when compared with fish subjected to MAP (5/70/25, O2/CO2/N2, respectively). Likewise, Leal Rodrigues *et al*. (2016) showed that TBARS value increased faster in samples subjected to MAP (80/20, CO2/N2, respectively) than in aerobic packaging and vacuum packaging during refrigerated storage (22 days at 4 °C) of rainbow trout (*Oncorhynchus mykiss*) fillets. Notably, Fagan *et al*. (2004) obtained contradictory results when storing freeze-
chilled mackerel and farmed salmon fillets under MAP conditions; thus, 100% CO2 MAP
led to the highest TBA and peroxide values for stored mackerel, while the lowest values
of these oxidation indices were revealed in the case of salmon storage. In agreement with
this result in freeze-chilled salmon, Masniyom et al. (2002) observed the lowest TBA
values in refrigerated (4 °C for 21 days) seabass (Lates calcarifer) slices that were
packaged under 100% CO2 MAP when compared with fish subjected to 60–80% CO2
MAP conditions. Similarly, Yesudhason et al. (2010) observed a lower TBARS
development during storage (25 days at 0-2 °C) of seer fish (Scomberomorus commerson)
steaks under CO2-enriched MAP (70/30, CO2/O2, respectively) when compared with air-
packaged fish. Later on, Sun et al. (2017) subjected swimming crab (Portunus
trituberculatus) to combined processing including MAP at different gas concentrations
and subsequent superchilled storage for 20 days; as a result, the lowest TBARS value was
detected in fish corresponding to 100% CO2 packaging.

CONCLUSIONS

The present research provides novel information concerning the employment of a
CO2 packaging and HPP combination on seafood. An additional preserving effect on lipid
quality is concluded for the current combination according to their complementary
barriers. Thus, previous HPP minimises the effect of endogenous enzyme activity (i.e.,
lipid hydrolysis and oxidation) during refrigerated storage, while CO2-enriched
packaging minimises the development of lipid oxidation resulting from the presence of
O2 in the packaging medium. As a remarkable advantage of this combination processing,
a lowering of the pressure-level requirement is likely to be carried out. Consequently,
further research is envisaged to optimise the current process combination. For it,
complementary sensory and physical determinations would be necessary to fulfil
consumer’s demand for high-quality fresh products that have been minimally processed
and do not include the presence of added chemical preservatives. The proposed approach should consider the international regulations concerning the employment of MAP and HPP for seafood while taking into account commercial and practical considerations to also agree with the industrial needs and capacities.

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Ethical Guidelines

Ethics approval was not required for this research.
REFERENCES


Annotated References

1 Amanatidou et al. (2000). This study represents the first approach for employing MAP/HPP as a combined processing on seafood. On the basis of the conditions employed, a definite effect could not be concluded on lipid oxidation development.

2 Aubourg et al. (2010). This study showed the need for employing relatively soft HPP conditions on Coho salmon (O. kisutch). It served as a basis for searching an accurate combination of HPP with CO2 packaging.

3 Fagan et al. (2004). This work showed the interest of employing a CO2-enriched MAP on salmon. Thus, a decreased TBARS formation was obtained by applying a 100%-CO2 atmosphere.

4 Pérez-Won et al. (2020). This study provided the basis for the current study. In it, the effect of several CO2-packaging and HPP combinations on microbial and physicochemical properties of refrigerated salmon (O. kisutch) were analysed.

5 Vinagre et al. (2011). This work reviewed the nutritional and technological possibilities of the current salmon (O. kisutch) species. In it, the need for novel and advanced processing was found mandatory to obtain high-quality products.
Coho salmon (*Oncorhynchus kisutch*)

- **CO₂ packaging**
- **High-pressure processing (HPP) (150 MPa, 5 min)**

Refrigerated storage (4 °C)

- **Assessment of lipid hydrolysis**
- **Determination of lipid oxidation**

Inhibitory effect on lipid hydrolysis and oxidation by combined CO₂ packaging and HPP

**Graphical Abstract**
<table>
<thead>
<tr>
<th>Treatment condition</th>
<th>Refrigeration time (days)</th>
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<td>48.86 bC (0.82)</td>
<td>55.02 cC (3.61)</td>
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</tbody>
</table>

* Average values of three replicates (n=3); standard deviations are indicated in brackets. In the same column, average values followed by different low-case letters (a-d) indicate significant differences ($p<0.05$) as a result of the treatment condition. In the same row, average values followed by capital letters (A-D) indicate significant differences ($p<0.05$) as a result of the refrigeration time.

** Treatment conditions: B-0 (fish without CO$_2$ packaging and not subjected to HPP; control fish), B-1 (fish packaged under CO$_2$ condition and not subjected to HPP), B-2 (fish subjected to HPP without CO$_2$ packaging) and B-3 (fish subjected to CO$_2$ packaging and HPP).
TABLE 2
Conjugated dienes content* in refrigerated salmon muscle subjected to CO2-enriched packaging and 150-MPa high-pressure processing (HPP)**

<table>
<thead>
<tr>
<th>Treatment condition</th>
<th>Refrigeration time (days)</th>
<th>0 (Initial raw fish)</th>
<th>3</th>
<th>7</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-0</td>
<td></td>
<td>0.21 A (0.01)</td>
<td>0.19 aA (0.07)</td>
<td>0.27 aB (0.03)</td>
<td>0.34 bB (0.03)</td>
</tr>
<tr>
<td>B-1</td>
<td></td>
<td>0.21 A (0.01)</td>
<td>0.26 aB (0.01)</td>
<td>0.27 aB (0.05)</td>
<td>0.26 aB (0.03)</td>
</tr>
<tr>
<td>B-2</td>
<td></td>
<td>0.21 A (0.01)</td>
<td>0.27 aB (0.03)</td>
<td>0.31 aB (0.02)</td>
<td>0.35 bB (0.02)</td>
</tr>
<tr>
<td>B-3</td>
<td></td>
<td>0.21 A (0.01)</td>
<td>0.25 aA (0.04)</td>
<td>0.25 aA (0.05)</td>
<td>0.24 aA (0.04)</td>
</tr>
</tbody>
</table>

* Data calculation as expressed in the Material and Methods section. In the same column, average values followed by different low-case letters (a-b) indicate significant differences ($p<0.05$) as a result of the treatment condition. In the same row, average values followed by capital letters (A-B) indicate significant differences ($p<0.05$) as a result of the refrigeration time.

** Treatment conditions as expressed in Table 1.
### TABLE 3

Peroxide value (meq. active oxygen·kg⁻¹ lipids) in refrigerated salmon muscle subjected to CO₂-enriched packaging and 150-MPa high-pressure processing (HPP)**

<table>
<thead>
<tr>
<th>Treatment condition</th>
<th>Refrigeration time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 (Initial raw fish)</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>B-0</td>
<td>1.60 A (0.37)</td>
</tr>
<tr>
<td>B-1</td>
<td>1.60 A (0.37)</td>
</tr>
<tr>
<td>B-2</td>
<td>1.60 A (0.37)</td>
</tr>
<tr>
<td>B-3</td>
<td>1.60 AB (0.37)</td>
</tr>
</tbody>
</table>

* Average values of three replicates (n=3); standard deviations are indicated in brackets. In the same column, average values followed by different low-case letters (a-d) indicate significant differences (p<0.05) as a result of the treatment condition. In the same row, average values followed by capital letters (A-C) indicate significant differences (p<0.05) as a result of the refrigeration time.

** Treatment conditions as expressed in Table 1.
<table>
<thead>
<tr>
<th>Treatment condition</th>
<th>Refrigeration time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 (Initial raw fish)</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>B-0</td>
<td>1.32 A (0.25)</td>
</tr>
<tr>
<td>B-1</td>
<td>1.32 A (0.25)</td>
</tr>
<tr>
<td>B-2</td>
<td>1.32 A (0.25)</td>
</tr>
<tr>
<td>B-3</td>
<td>1.32 A (0.25)</td>
</tr>
</tbody>
</table>

* Average values of three replicates (n=3); standard deviations are indicated in brackets. In the same column, average values followed by different low-case letters (a-c) indicate significant differences (p<0.05) as a result of the treatment condition. In the same row, average values followed by capital letters (A-D) indicate significant differences (p<0.05) as a result of the refrigeration time.

** Treatment conditions as expressed in Table 1.
### TABLE 5

Fluorescence ratio value in refrigerated salmon muscle subjected to CO2-enriched packaging and 150-MPa high-pressure processing (HPP)**

<table>
<thead>
<tr>
<th>Treatment condition</th>
<th>Refrigeration time (days)</th>
<th>0 (Initial raw fish)</th>
<th>3</th>
<th>7</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.97 A (0.10)</td>
<td>2.59 bB (0.18)</td>
<td>3.88 bC (0.17)</td>
<td>5.56 cD (0.91)</td>
</tr>
<tr>
<td>B-0</td>
<td></td>
<td>0.97 A (0.10)</td>
<td>1.33 aB (0.06)</td>
<td>1.69 aC (0.16)</td>
<td>2.70 bD (0.04)</td>
</tr>
<tr>
<td>B-1</td>
<td></td>
<td>0.97 A (0.10)</td>
<td>2.54 bB (0.02)</td>
<td>3.70 bC (0.04)</td>
<td>5.00 cD (0.16)</td>
</tr>
<tr>
<td>B-2</td>
<td></td>
<td>0.97 A (0.10)</td>
<td>1.21 aA (0.13)</td>
<td>1.68 aB (0.02)</td>
<td>2.50 aC (0.09)</td>
</tr>
<tr>
<td>B-3</td>
<td></td>
<td>0.97 A (0.10)</td>
<td>1.32 aA (0.13)</td>
<td>1.69 aB (0.02)</td>
<td>2.70 bD (0.04)</td>
</tr>
</tbody>
</table>

* Data calculation as expressed in the Material and Methods section. In the same column, average values followed by different low-case letters (a-c) indicate significant differences ($p<0.05$) as a result of the treatment condition. In the same row, average values followed by capital letters (A-D) indicate significant differences ($p<0.05$) as a result of the refrigeration time.

** Treatment conditions as expressed in Table 1.