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Application of high hydrostatic pressures for improving the quality of frozen albacore (*Thunnus alalunga*)

Aplicación de altas presiones hidrostáticas para la mejora de la calidad del atún blanco (*Thunnus alalunga*) congelado

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RESUMEN

El atún blanco o bonito del norte (*Thunnus alalunga*) es un pescado muy apreciado por su calidad nutricional (alto contenido en ácidos grasos insaturados, proteína de alto valor biológico) y sus excelentes características organolépticas. Al final de la primavera, los individuos jóvenes inician su migración hacia el Golfo de Bizkaia en busca de alimento, lo que da inicio a la costera del bonito del norte, que comienza en junio y finaliza en octubre. Es en este periodo en el que el bonito del norte es capturado por las flotas pesqueras vascas. La pesca del bonito del norte tiene una gran importancia socio-económica en el País Vasco. Concretamente, en 2018, la flota pesquera vasca capturó 8.448 toneladas de atún blanco, lo que constituyó el 29,3 % de las capturas totales llevadas a cabo por barcos procedentes de los países miembros de la Unión Europea (UE-28). Sin embargo, la alta estacionalidad de esta especie pesquera, unida a su limitada vida útil, condicionan su disponibilidad en el mercado.

Todo ello hace necesario aplicar métodos de conservación que garanti su disponibilidad en el mercado durante todo el año. La congelación es un método de conservación que se ha utilizado para el almacenamiento a lo largo plazo de numerosas especies pesqueras. Sin embargo, la desnaturalización proteica, la oxidación lipídica y los daños estructurales que se producen en el tejido muscular como consecuencia de la congelación conllevan una serie de cambios que afectan negativamente a la calidad del pescado congelado, entre los cuales destaca la pérdida de peso porque implica importantes pérdidas económicas para la industria pesquera.

En las últimas décadas, se ha propuesto la utilización de tecnologías emergentes para minimizar la pérdida de la calidad provocada por el proceso de congelación y descongelación en los productos pesqueros, tales como la aplicación de altas presiones, ultrasonidos o campos magnéticos. Sin embargo, el alto coste que conlleva la utilización de estas tecnologías ha limitado su implementación a nivel industrial.

En los últimos años se ha estudiado la aplicación de pretratamientos por altas presiones hidrostáticas (APH) antes de la congelación para mejorar la calidad de productos pesqueros congelados, mostrando resultados prometedores. El tratamiento por altas presiones hidrostáticas es una tecnología no-térmica que se ha utilizado como alternativa a los tratamientos convencionales para conservar numerosos alimentos, ya que permite

alargar su vida útil sin afectar negativamente a la calidad nutricional y sensorial de la forma en la que lo hacen los tratamientos convencionales de conservación. Esto se debe a que el tratamiento por altas presiones se rige por la Ley de Le Châtelier, que consiste en que todas las reacciones que implican una reducción de volumen se ven favorecidas, mientas que aquellas que implican un aumento del volumen son inhibidas. Por lo tanto, a pesar de que la presión puede conllevar cambios en la estructura de algunas macromoléculas tales como las proteínas o las enzimas, los compuestos de bajo peso molecular responsables de la calidad nutricional y sensorial del alimento, tales como las vitaminas, los minerales o los compuestos aromáticos, no se ven afectados por la presión. Además, de acuerdo al Principio Isostático, la presión se transmite de forma uniforme e instantánea en la totalidad del alimento, por lo que la eficacia del tratamiento APH es independiente del tamaño, la forma o el volumen del alimento, al contrario que los tratamientos térmicos.

 No obstante, los tratamientos por altas presiones conllevan una serie de cambios en la estructura de las proteínas y en los lípidos del pescado que pueden dan lugar a cambios en algunas características sensoriales del pescado, tales como el color o la textura o la capacidad del tejido muscular para retener agua. En ese sentido, algunos estudios recientes han demostrado que el tratamiento por altas presiones permite mejorar la retención de agua en productos frescos. Tal y como se ha detallado anteriormente, la pérdida de agua es una de las principales limitaciones de la congelación en productos pesqueros. El grado en el que estos cambios en la calidad se producen depende del nivel de presión, el tiempo durante el cual se aplica esa presión y la temperatura a la que se lleva a cabo el tratamiento. Por tanto, es fundamental optimizar esos tres parámetros para lograr una mejora de la calidad en el producto.

Por todo ello, en esta tesis se planteó la utilización de pretratamientos por APH con el objeto de mejorar la calidad del pescado congelado, especialmente para disminuir las pérdidas de peso. Para ello, se evaluaron las modificaciones en las pérdidas de peso (pérdidas de peso asociadas a la descongelación, perdidas de peso por el cocinado y pérdidas de peso totales), el color (sistema CIELab), la textura (análisis de perfil de textura), el contenido en proteína soluble en solución salina y la oxidación lipídica (sustancias reactivas al ácido tiobarbitúrico, índice TBA) como consecuencia del tratamiento por APH.

Se comenzó por evaluar el impacto del tratamiento por APH en la calidad del atún blanco fresco (no congelado) aplicando un amplio rango de presiones (50–500 MPa durante 2 min). A partir de 200 MPa se produjo una reducción en la pérdida de peso. Sin embargo, a medida que aumentaba la presión, los cambios en el color, la apariencia y la textura se iban acentuando, comenzando a ser apreciables a partir de 200–250 MPa en comparación con las muestras no tratadas. Más concretamente, el tratamiento por APH dio lugar a un aumento gradual de los valores L^* (luminosidad) y b^* (rango amarillo–azul), así como en la dureza y la masticabilidad a medida que aumentaba la presión. Además, se observó una disminución progresiva del contenido en proteína soluble en solución salina a medida que aumentaba la presión. En relación a la oxidación lipídica, no se observaron cambios en el índice TBA respecto a las muestras no tratadas como consecuencia del tratamiento por APH. Por tanto, los tratamientos a 200–250 MPa durante 2 min fueron los más adecuados para reducir las pérdidas de peso y mantener una calidad lo más parecida posible a la del pescado fresco.

Una vez conocido el rango de presiones idóneo para reducir las pérdidas de peso en el pescado fresco, se procedió a evaluar el impacto del pretratamiento por APH en la calidad del atún blanco congelado:

En primer lugar, se evaluó el impacto del pretratamiento por APH antes de congelación en el atún blanco sometido a un proceso de congelación y descongelación. El rango de presiones utilizadas (200–300 MPa durante 0–6 min) se seleccionó en base a los resultados obtenidos en el atún blanco fresco. Los tratamientos a 200 MPa durante 6 min fueron los que permitieron reducir en mayor medida las pérdidas de peso asociadas a la descongelación y por cocinado (disminuciones del 53,7 % y del 55,4 % respectivamente, en comparación con las muestras no tratadas). La mayor reducción de las pérdidas de peso totales respecto al control también se produjo en las muestras tratadas a 200 MPa durante 6 min (disminución del 51,0 % respecto a las muestras no tratadas). Sin embargo, estos tratamientos dieron lugar a cambios en el color (valores L^* y b^* significativamente mayores que en las muestras no tratadas) y en la textura (adhesividad y elasticidad significativamente mayores que en las muestras no tratadas), así como en el contenido en proteína soluble en solución salina (menor contenido que en las muestras no tratadas). En cuanto al índice TBA, al igual que en el atún blanco fresco, no se observaron cambios como consecuencia del pretratamiento por altas presiones a 200 MPa durante 6 min. El

pretratamiento a 200 MPa durante 2 min permitió reducir las pérdidas de peso tanto asociadas a la descongelación, como por cocinado, así como las pérdidas de peso totales, sin dar lugar a cambios apreciables en el color.

En segundo lugar, se evaluó el potencial del pretratamiento por APH (200 MPa durante 0–6 min) antes de la congelación para mejorar la calidad en el atún blanco almacenado en congelación durante tiempos prolongados (hasta 12 meses). Transcurridos 12 meses de almacenamiento en congelación, el pretratamiento a 200 MPa durante 6 min permitió mantener unas pérdidas de peso asociadas a la descongelación similares a las del pescado fresco no tratado, mientras que en las muestras no tratadas por APH estas aumentaron un 55,0 % respecto al pescado fresco (no congelado). Además, a tiempos de almacenamiento prolongados (a partir de 6 meses), este pretratamiento implicó la inhibición de la oxidación lipídica que se produce durante almacenamiento en congelación. Sin embargo, también dio lugar a cambios notables tanto en el color (aumento de los valores $L*$ y $b*$ respecto a las muestras no tratadas) como en la textura (aumento de la adhesividad en comparación con las muestras no tratadas) del pescado. Tras el cocinado de las muestras, las diferencias en el color desaparecieron. Por tanto, el pretratamiento por altas presiones antes de la congelación permitiría mejorar la calidad del atún blanco congelado, y más concretamente, disminuir las pérdidas de peso que se producen como consecuencia del proceso de congelación y descongelación, especialmente cuando este va a ser consumido una vez cocinado.

En tercer lugar, se evaluó la aplicación de los pretratamientos por APH (200 MPa durante 6 min y 600 MPa durante 0 min) antes del almacenamiento en congelación y antes de la descongelación con el objetivo de mejorar la calidad del atún blanco congelado. Tanto el pretratamiento antes del almacenamiento en congelación como el pretratamiento antes de la descongelación permitieron reducir las pérdidas de peso asociadas a la descongelación en comparación con las muestras no tratadas. El pretratamiento a 200 MPa durante 6 min antes de la descongelación permitió mantener estables las pérdidas de peso asociadas a la descongelación durante 45 días de almacenamiento en congelación. Los cambios en el color y la textura provocados por la presión fueron similares al aplicar el pretratamiento antes del almacenamiento en congelación o antes de la descongelación. Los pretratamientos a 600 MPa durante 0 min redujeron las pérdidas de peso asociadas a la descongelación en mayor medida que a 200 MPa durante 6 min, aunque los cambios en

el color y la textura inducidos por la presión también fueron más acusados. Por tanto, el pretratamiento a 200 MPa durante 6 min antes de la descongelación fue el más adecuado para disminuir las pérdidas de peso asociadas a la descongelación y mantener una calidad parecida a la del atún blanco fresco.

Por último, se evaluó el efecto de diferentes condiciones de congelación (temperatura: –20 ℃, velocidad de aire: 1 m s^{-1}; –20 ℃, 5 m s^{-1}; –50 ℃, 1 m s^{-1}; –50 ℃, 5 m s^{-1}) en la calidad del atún blanco previamente tratado por APH (200 MPa durante 6 min). Las muestras se mantuvieron almacenadas en congelación durante 2 y 9 meses, tras los cuales fueron descongeladas y analizadas. El efecto del pretratamiento por altas presiones en la calidad del pescado fue más importante que el de las condiciones de congelación. En todas las muestras pretratadas por APH se observó una reducción similar de las pérdidas de peso asociadas a la descongelación (en torno al 40 % respecto a sus respectivos controles) independientemente de la temperatura y la velocidad de aire utilizadas durante la congelación de las muestras. No se observaron diferencias en el color debidas a la temperatura y la velocidad de aire utilizadas durante la congelación. En relación a la textura, tan solo se observaron diferencias provocadas por el tipo de congelación aplicada en la dureza, que fue más próxima a la del pescado fresco en las muestras congeladas a una temperatura de –50 ℃ que en aquellas congeladas a –20 ℃ y 1 m s^{-1}. Por lo tanto, el pretratamiento por altas presiones a 200 MPa durante 6 min seguido de una congelación a –50 ℃ (ya sea a una velocidad de aire de 1 o de 5 m^{-1}) sería adecuado para lograr una reducción de las pérdidas de peso asociadas a la descongelación durante hasta 9 meses de almacenamiento en congelación.

Al comparar el efecto de los pretratamientos por altas presiones antes de la congelación, antes del almacenamiento en congelación y antes de la descongelación, se observó que todos los pretratamientos a 200 MPa durante 6 min fueron efectivos para reducir las pérdidas de peso asociadas a la descongelación. Sin embargo, los cambios en el color y la textura inducidos por la presión fueron más acusados cuando el pretratamiento se aplicó antes del almacenamiento en congelación y de la descongelación que cuando se aplicó antes de la congelación.

A modo de conclusión, el pretratamiento por APH es una tecnología de gran utilidad para reducir las pérdidas de peso producidas durante el procesado y el almacenamiento del pescado congelado.

ABSTRACT

Albacore (*Thunnus alalunga*) is very appreciated thanks to its nutritional quality (high content of unsaturated fatty acids, high biological value proteins) and its excellent organoleptic characteristics. In late spring, juveniles begin their feeding migration towards the Bay of Biscay. This fact involves the beginning of the albacore season in the Basque Country, which starts in June and ends in October. During that period, albacore is caught by Basque fisheries. Albacore fisheries have great socio-economic importance in the Basque Country. More specifically, in 2018 the Basque fisheries caught 8,448 tons of albacore, which represents the 29.3% of the total catches by European fishing fleets (EU-28). However, its high seasonality and its limited shelf life affect its market availability.

Hence, it is necessary to apply preservation methods that ensure its market availability throughout the year. Freezing has been used for the long-term storage of several seafood species. However, protein denaturation, lipid oxidation and structural damage to muscle tissue as a consequence of freezing and frozen storage lead to several changes that negatively affect the quality of frozen fish. Among these negative changes, weight loss can be highlighted because it involves important economic losses for the seafood industry.

Over the last decades, several emerging technologies have been proposed to minimize the quality loss caused by the freeze-thaw process in seafood products, such as the application of high-pressures, ultrasounds or magnetic fields. However, the high cost that involves their application has limited their industrial implementation.

Recently, high hydrostatic pressure (HHP) has shown great potential for improving the quality of frozen seafood products. HPP treatment is a non-thermal technology that has been used as an alternative to conventional food preservation methods. It allows to extend the shelf-life of food without negatively affecting its nutritional and sensorial quality. This is caused by the fact that HPP treatment is governed by the Le Châtelier's Principle, which states that under equilibrium conditions all reactions that involve a volume reduction are favored when the pressure increases, whereas those reactions that involve a volume increment are inhibited. Therefore, despite the fact that pressure can lead to changes in the structure of some macromolecules such as proteins or enzymes, low-weight compounds such as vitamins, minerals or aromatic compounds are not affected by

pressure. Furthermore, in accordance to Isostatic Principle, pressure is transferred uniformly and instantaneously to the entire food. Consequently, the effectiveness of high-pressure treatments is not dependent on size, shape or volume of sample, in contrast to thermal treatments.

High-pressure treatments involve changes in protein structure as well as in lipids that can lead to several changes in color, texture or water retention of muscle tissue. In this sense, some authors have recently shown that high-pressure treatments allow the improvement of the water retention ability in fresh products. As mentioned before, water loss is one of the main limitations of frozen fish products. Since the extent of quality changes depends on the pressure level, the pressurization time and the temperature of the treatment, it is essential to optimize these parameters in order to achieve higher quality.

Therefore, in this thesis, HPP pretreatments were proposed for improving the quality of frozen fish, especially for the reduction of weight losses. To achieve this aim, changes in weight losses (thawing loss, cooking loss and total weight loss), color (CIELab system), texture (texture profile analysis; TPA), salt-soluble protein content and lipid oxidation (TBARS value) as a consequence of HPP treatments were assessed.

First of all, the impact of HPP treatment on the quality of fresh albacore by applying a wide range of pressures (50–500 MPa for 2 min) was evaluated. Pressure induced some changes in color and texture of albacore samples, which were more pronounced as the pressure increased. Weight losses started to decrease from 200 MPa. However, changes in color, appearance and texture were more pronounced as the pressure increased, being appreciable from 200–250 MPa in comparison to non-treated samples. More specifically, HPP treatment led to a progressive increase of $L*$ (luminosity) and $b*$ value (yellow-blue range) as well as in hardness and chewiness. There was also a decrease in salt-soluble protein content as the pressure increased. Regarding lipid oxidation, there were no changes in TBARS value with respect to the non-treated samples as a result of HPP treatment. Thus, HPP treatments at 200–250 MPa were optimal for reducing weight losses while retaining fresh-like quality.

Once that the range of pressures that allowed the reduction of weight losses was found, the impact of HPP pretreatment on the quality of frozen albacore was evaluated:

Firstly, the impact of HPP pretreatment before freezing on albacore after the freeze-thawing process was evaluated. The range of pressures applied (200–300 MPa for 0–6 min) was selected from the results obtained in fresh albacore. The greatest reduction in thawing and cooking losses was observed in HPP-pretreated albacore at 200 MPa for 6 min (53.7 and 55.4% decrease with respect to non-treated samples, respectively). The Samples treated at 200 MPa for 6 min also showed the greatest reduction of total weight loss (51.0% decrease with respect to non-treated samples). However, this pretreatment resulted in noticeable changes in color ($L*$ and $b*$ values were significantly higher than in the non-treated samples) and texture (adhesiveness and springiness were significantly higher than in non-treated samples), as well as in salt-soluble protein content (lower content than non-treated samples). As in fresh albacore, there were no changes in TBARS value as a consequence of HPP pretreatments at 200 MPa for 6 min. HPP pretreatment at 200 MPa for 2 min decreased thawing, cooking and total weight losses without leading to appreciable changes in color.

Secondly, the potential of HPP pretreatment (200 MPa for 0–6 min) before freezing for improving the quality of frozen albacore after long-term storage (up to 12 months) was evaluated. After 12 months of frozen storage, thawing losses of HPP-pretreated albacore at 200 MPa for 6 min were similar to those found in non-treated fresh fish samples, whereas in non-treated samples they were 55.0 % higher than in the fresh samples. Furthermore, after long-term storage times (from 6 months) this pretreatment involved the inhibition of lipid oxidation inherent to the frozen storage. However, this pretreatment induced some changes in color (higher $L*$ and $b*$ values than non-treated samples) and texture (higher adhesiveness than non-treated samples) properties of albacore. After cooking, there were no differences in color between the pretreated samples and the control ones. Thus, HPP pretreatment is a suitable technology to improve the quality of frozen albacore, and more specifically, to decrease the thawing loss, especially when consumed once cooked.

Thirdly, the application of HPP (200 MPa for 6 min and 600 MPa for 0 min) before frozen storage or before thawing was evaluated in order to improve the quality of frozen albacore. Both pretreatments allowed the reduction of thawing loss in comparison with their respective controls. HPP pretreatment at 200 MPa for 6 min before thawing allowed to maintain thawing loss stable during up to 45 days of frozen storage. Color and texture

changes induced by pressure were similar when HPP pretreatment was applied before frozen storage or before thawing. HPP pretreatments at 600 MPa for 0 min led to a higher decrease of thawing loss than 200 MPa for 6 min, although color and texture changes induced by pressure were also higher in samples treated at 600 MPa for 0 min. Thus, 200 MPa for 6 min before thawing was the best HPP pretreatment to decrease thawing loss while retaining fresh-like quality.

Fourthly, the effect of different freezing conditions (temperature: –20 ℃, air velocity: 1 m s^{-1}; –20 ℃, 5 m s^{-1}; –50 ℃, 1 m s^{-1}; –50 ℃, 5 m s^{-1}) on the quality of HPP-pretreated (200 MPa for 6 min) albacore was evaluated. After 2 and 9 months of frozen storage, samples were thawed and analyzed. The effect of HPP pretreatment at 200 MPa for 6 min was more important than the effect of freezing conditions. All HPP-pretreated samples showed a similar thawing loss reduction (around 40% with respect to their respective controls) regardless of the temperature and the air velocity used during the freezing process. There were no differences in color as a consequence of freezing conditions. Regarding the texture, differences were only found in hardness, which was more akin to fresh fish in samples frozen at –50 ℃ than in samples frozen at –20 ℃ and 1 m s^{-1}. Thus, HPP pretreatment at 200 MPa for 6 min followed by freezing at –50 ℃ (regardless of the air velocity) would be suitable to achieve the decrease of thawing loss during up to 9 months of frozen storage.

All HPP pretreatments (before freezing, before frozen storage and before thawing) at 200 MPa for 6 min allowed the achievement of a reduction of thawing loss during frozen storage. However, changes in color and texture were more pronounced in HPP pretreatments applied before frozen storage or before thawing than in those applied before freezing.

In conclusion, HPP pretreatment is a suitable technology to decrease weight losses during the processing and storage of frozen fish.

ABBREVIATIONS

Δ**E**	Total color difference/ Diferencia total de color
20/1	Chamber temperature/Temperatura de la cámara: –20 ℃; air velocity/velocidad de aire: 1 m s^{-1}
20/5	Chamber temperature/Temperatura de la cámara: –20 ℃; air velocity/velocidad de aire: 5 m s^{-1}
50/1	Chamber temperature/Temperatura de la cámara: –50 ℃; air velocity/velocidad de aire: 1 m s^{-1}
50/5	Chamber temperature/Temperatura de la cámara: –50 ℃; air velocity/velocidad de aire: 5 m s^{-1}
a*	red-greenness/rango rojo-verde
ANOVA	Analysis of variance/Análisis de la varianza
b*	yellow-blueness/rango amarillo-azul
BFS	Before frozen storage/Antes del almacenamiento en congelación
BT	Before thawing/Antes de la descongelación
CI	Confidence Interval/Intervalo de confianza
CIE	International Commission on Illumination/Comisión Internacional en Iluminación
DHA (C22:6n-3)	docosahexaenoic acid/ácido docosahexaenoico
DMA	Dimethylamine/dimetilamina
EDTA	Ethylenediaminetetraacetic acid/Ácido etilendiaminotetraacético
EPA (C20:5n-3)	eicosapentaenoic acid/ácido eicosapentaenoico
FA	Formaldehyde/Formaldehído
FAO	Food and Agriculture Organization/ Organización de las Naciones Unidas de la Alimentación y la Agricultura
FFA	Free fatty acids/Ácidos grasos libres
HPP/APH	High-pressure processing/Altas presiones hidrostáticas
ICCAT	International Commission for the Conservation of Atlantic Tunas/Comisión Internacional para la Conservación del Atún Atlántico
ISSCAAP	International Standard Statistical Classification for Aquatic Animals and Plants/Clasificación Estadística Internacional Uniforme de los Animales y Plantas Acuáticos

KCl	Potassium chloride/Cloruro de potasio
L*	Lightness/Luminosidad
MDA	Malonaldehyde/Malondialdehído
PUFA	Polyunsaturated fatty acid/Ácido graso poliinsaturado
rpm	Revolutions per minute/Revoluciones por minuto
TBA	Thiobarbituric acid/Ácido tiobarbitúrico
TBARS	Thiobarbituric acid reactive substances/Sustancias reactivas al ácido tiobarbitúrico
TCA	Trichloroacetic acid/Ácido tricloroacético
TPA	Texture profile analysis/Análisis de Perfil de Textura
TEP	1,1,3,3-tetraethoxypropane/1,1,3,3-tetraetoxipropano
TMAO	Trimethylamine oxide/Óxido de trimetilamina
TMAOase	trimethylamine-oxide aldolase/óxido de trimetilamina aldolasa
EU-28/UE-28	European Union (28 member countries)/Unión Europea (28 paíeses miembros)
WHC	Water holding capacity/Capacidad de retención de agua

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1. INTRODUCTION

Fish demand is increasing worldwide because its consumption is related to a healthy diet. Regarding the Basque Country, the Basque fish industry is a European referent in terms of both fish production and fish processing. Among all fish species that are produced and processed in the Basque Country, albacore (*Thunnus alalunga*) has particular interest because of its high nutritional quality as well as its excellent sensorial properties. Albacore has also an important place in the Basque gastronomy, since it is the main ingredient of some traditional Basque dishes.

Despite its high demand, fish is a high perishable food product. After fish death, microbial and endogenous enzyme activities as well as chemical reactions lead to a progressive quality loss. Hence, it is necessary to apply preservation methods that allow to extend its shelf life. Frozen storage is one of the most widely used preservation methods for long-term preservation of fish products. However, several changes that take place during freezing and frozen storage may affect the fish quality, such as protein denaturation, lipid oxidation or weight loss (Gökoğlu & Yerlikaya, 2015b). In this sense, weight loss is one of the most important issues of frozen fish products because it leads to economic losses for fish industry, as well as the loss of some proteins and vitamins (Gökoğlu & Yerlikaya, 2015b).

During last decades, research has been focused on the use of innovative technologies for food processing and preservation, such as pulsed electric fields, ultrasounds or high-pressures (Astráin-Redín et al., 2021; Barba et al., 2015a; Misra et al., 2017). Among these technologies, high-pressure processing (HPP) can be highlighted because it allows shelf-life extension without affecting nutritional and sensorial properties of fish products. HPP may also be used as a pretreatment before freezing for improving the quality of frozen fish (Torres et al., 2014; Truong et al., 2016; Vázquez et al., 2013, 2018).

In this chapter some general aspects related to albacore are detailed. A brief description of freezing and frozen storage and high-pressure, as well as the respective changes that they involve in the fish quality are also described.

1.1. Albacore (*Thunnus alalunga*)

1.1.1. Classification and names

Albacore belongs to the family of scombrids and to the genus *Thunnus* and its scientific name is *Thunnus alalunga*, which was first described by Bonnaterre in 1788.

According to Collette & Nauen (1983), the scientific classification of albacore is:

Phylum: Chordata

    Subphylum: Vertebrata

        Class: Osteichthyes

            Subclass: Actinopterygii

                Order: Perciformes

                    Suborder: Scombroidei

                        Family: Scombridae

                            Genus: *Thunnus*

                                Species: *alalunga*

According to ICCAT (International Commission for the Conservation of Atlantic Tunas), the official names of this species are albacore (English), germon (French) and atún blanco (Spanish) (Froese & Pauly, 2019; ICCAT, 2005).

In accordance with the Food and Agriculture Organization (FAO), albacore belongs to group 36 of International Standard Statistical Classification for Aquatic Animals and Plants (ISSCAAP), which includes tunas, bonitos and billfishes (Froese & Pauly, 2019).

1.1.2. Morphological characteristics

Albacore has a fusiform body. The body is dark blue on the dorsal side and silvery-white on the ventral part, being both parts bounded by a lateral iridescent blue band in live fish (González-Garcés Santiso, 2003; ICCAT, 2005; Ortiz de Zárate Vidal, 2018).

It has a long pectoral fin (up to 30% length in fishes longer than 50 cm), 7–9 dorsal finlets, 7–8 anal finlets, two dorsal fins, and a short and crescent-shaped caudal fin (González-Garcés Santiso, 2003; ICCAT, 2005). The second dorsal fin is smaller and lighter than

the first one. The color of the anal fin is similar to the second dorsal one, although it has a white border.

1.1.3. Nutritional composition

Albacore is distinguished by its high nutritional quality, mainly by its fat composition.

Albacore is characterized by its high content in fat (6%) (Oehlenschläger, 2014). Albacore muscle has a high content of fat (6 %), and particularly of poly-unsaturated fatty acids (PUFAs) (> 2 g/100 g), mainly docosahexaenoic acid (DHA, C22:6n-3) and eicosapentaenoic acid (EPA, C20:5n-3). These fatty acids are highly beneficial to human health (Gökoğlu & Yerlikaya, 2015a; Oehlenschläger, 2014). Furthermore, its content in cholesterol is low (Oehlenschläger, 2014).

Albacore has high protein content (around 25 %) (Ben-Gigirey et al., 1999). Protein of albacore muscle is rich in essential amino acids, and thus, it has a high biological value (Oehlenschläger, 2014). The low connective tissue content (1–2%) makes it easy to digest (Oehlenschläger, 2014).

Albacore is a rich source of vitamins B_{12}, B_6 and B_3 (niacin) and has a considerable amount of vitamin D (Oehlenschläger, 2014).

Related to minerals, albacore is an important source of selenium and iodine (Gökoğlu & Yerlikaya, 2015a) and has good levels of magnesium, phosphorus, potassium, calcium and iron (Gökoğlu & Yerlikaya, 2015a). Furthermore, its content in sodium is low (Venugopal, 2005a).

1.1.4. Geographical distribution and biology

Albacore is an oceanic, epi- and mesopelagic species. It lives in tempered and topical waters of Pacific, Indian and Atlantic oceans and also in the Mediterranean Sea (Nikolic et al., 2017). It is distributed between 60º N y el 60º S (Erauskin-Extramiana et al., 2019).

There are six stocks: North Atlantic, South Atlantic, Indian Ocean, North Pacific, South Pacific and Mediterranean (Chust et al., 2019; Nikolic et al., 2017). Most of the albacore caught by Spanish fishing fleets comes from the North Atlantic (FAO, 2019).

Albacore is a highly migratory species. Feeding migration of juveniles towards the Bay of Biscay determines the albacore fishing by Basque fishing fleets (González-Garcés

Santiso, 2003). It is therefore crucial to understand the migratory behavior of albacore in the North Atlantic.

During the winter months, juveniles and adults are in Central Atlantic, close to the Azores Islands (Sagarminaga & Arrizabalaga, 2014). In early spring, both juveniles and adults migrate northward and eastward, respectively.

Coinciding with the warming of water, juveniles migrate to the northeast in order to find food towards the Bay of Biscay and the Southwest of Ireland in June and July, where they remain in the surface waters and are exploited by European fishing fleets (Nikolic et al., 2017; Sagarminaga & Arrizabalaga, 2014). In September-October, coinciding with the cooling of surface waters, they return to the central Atlantic. This migration occurs annually until fishes reach sexual maturity (when they reach around 90 cm in length, which corresponds to the age of 5 years) (Nikolic et al., 2017). By contrast, adult albacores, which prefer warmer and deeper waters, migrate westward to reproduce until they reach the Sargasso Sea and the Gulf of Mexico. They spawn in these waters. In September they return to the Central Atlantic (Ortiz de Zárate Vidal, 2018; Sagarminaga & Arrizabalaga, 2014).

Albacore fishery is affected by the migration behavior of this fish species. This fact has important implications for its market availability throughout the year, which is restricted to a particular period of the year.

1.1.5. Production data

The global capture production of ISSCAAP group 36 species (tuna, bonitos and billfishes) has progressively increased from 1950 to the present (Figure 1) (FAO, 2019). The global capture production of albacore has also increased from 1950 (Figure 2), although the most pronounced increase occurred between 1950 and 1974 (FAO, 2019). According to FAO Global Capture Production Dataset (FAO, 2020), in 2018 the global capture production of ISSCAAP group 36 species was 7,913,008 tons, of which 226,082 tons corresponded to albacore catches.
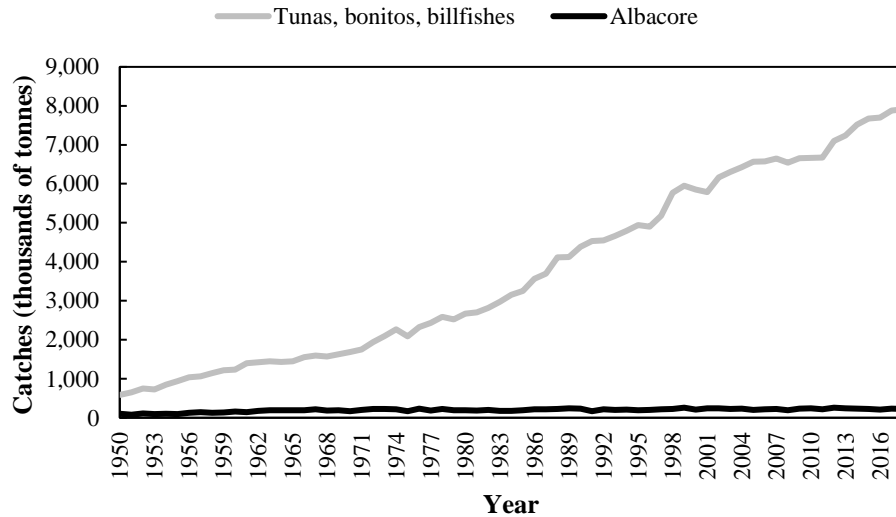
Figure 1. Global capture production (1950 – 2018) of ISSCAAP group 36 species. Own elaboration from FAO global capture production dataset (FAO, 2020).

According to FAO Global Capture Production Dataset (FAO, 2020), Spain is the fourth in the world in terms of albacore catches (7.5% of the global capture production in 2018) and the leading producer in the European Union (58.9% of total catches of European Union in 2018), followed by France (20.6%) and Ireland (10.8%).
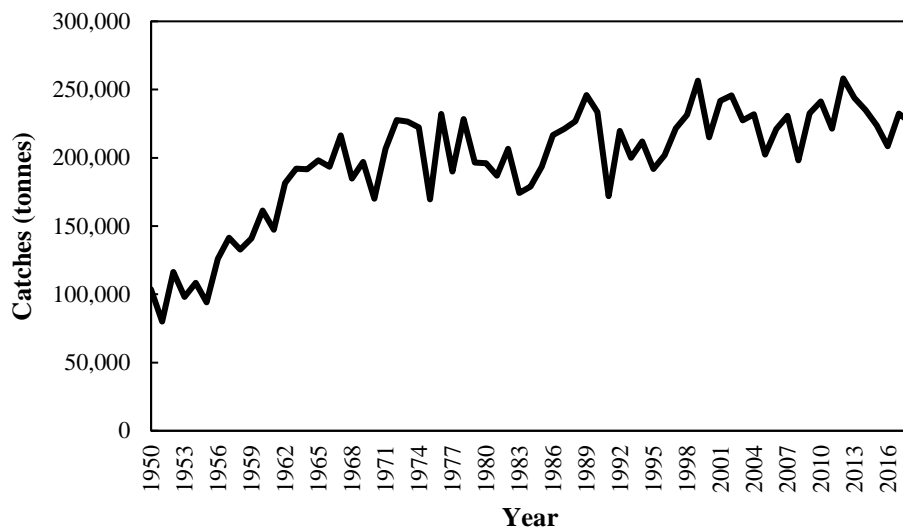
Figure 2. Global capture production (1950 – 2018) of ISSCAAP albacore. Own elaboration from FAO global capture production dataset (FAO, 2020).

The Basque Country is the largest producer in the State in terms of albacore catches. For instance, 15,840.2 tons of albacore were caught by Spanish fishing fleet in 2018 (Ministerio de Agricultura Pesca y Alimentación, 2020), of which 8,448 tons were caught by the Basque Country's fishing fleet (53.3% of the total catches in Spain) (Figure 3) (Ekonomiaren Garapen eta Azpiegitura Saila, 2020).
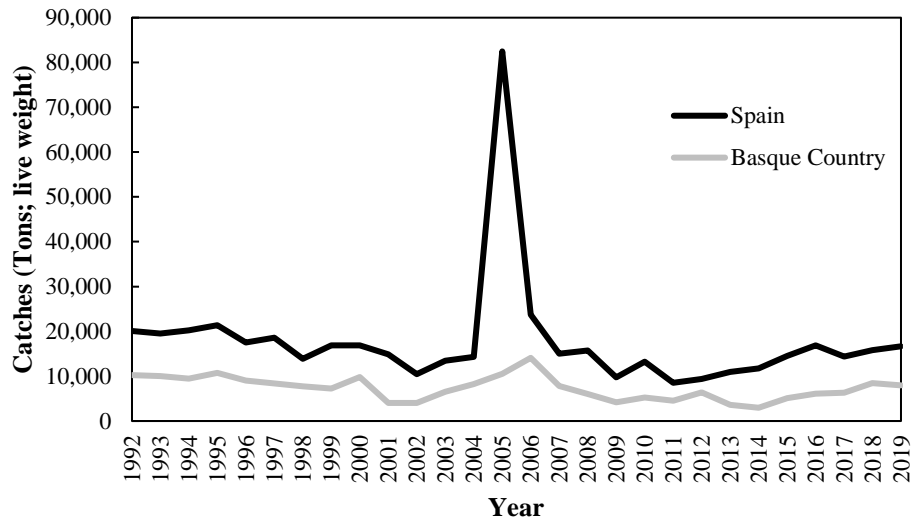
Figure 3. Capture production of albacore (1992 – 2019). Own elaboration from the database of the Ministry of Agriculture, Fisheries and Food of Spain (Ministerio de Agricultura Pesca y Alimentación, 2020) and by the statistical information provided by the Department of Agriculture, Fisheries and Food of the Basque Government (Ekonomiaren Garapen eta Azpiegitura Saila 2020).

1.1.6. Fishing quotes

The annual catches of albacore are regulated by fishing quotes, which are established every year by the ICCAT. In the European Union, fishing quotes are allocated between the Member States (Council Regulation (EEC) No 3760/92). These quotes avoid that albacore stocks are over-exploited.

From 2018, the albacore campaign has been prematurely closed due to the exhaustion of the Spanish fishing quota. For instance, in 2020 the albacore campaign was closed on August 19 despite the healty level of albacore stock in the Bay of Biscay (Secretaría General de Pesca, 2020). The great abundance of albacore individuals close to the Basque shore implies that the Basque fishing fleets do not need to move to Ireland to capture it and, consequently, the duration of the fishing trips are shorter. This fact limits the market avalaibility of albacore, that was restricted to a very short time.

1.1.7. Socio-economic importance

At state level, the Galician, Asturian, Cantabrian and Basque fishing fleets operate in the North Atlantic, whereas the Canary's fishing fleet operates in waters close to the archipelagos (González-Garcés Santiso, 2003).

Basque fishing fleets use two fishing gears: trolling line and baitboat in surface waters (up to 30 meters depth) (González-Garcés Santiso, 2003), coinciding with the summer migration of juvenile albacore to the Bay of Biscay and the Southwest of Ireland.

Regarding Basque Country, albacore is exploited by inshore fleet. In 2018, the inshore fleet of the Basque Country had 156 vessels and employed 1,131 people (7.25 crew members per vessel) (Ekonomiaren Garapen eta Azpiegitura Saila, 2019). Furthermore, there are 3.5 land-based workers for each sea-based worker (Baliño, 2016). Inshore fleet of the Basque Country catches anchovies from March to June and then albacore, between July and October. After the season for fishing albacore in the Bay of Biscay, sardine and mackerel become the target of inshore fleet (Iborra Martín, 2010).

The main species caught by Basque fishing fleet are anchovies, albacores, sardines, mackerels, hakes and bluefin tunas (Ekonomiaren Garapen eta Azpiegitura Saila, 2019). In 2018 the albacore catches represented 15.75% of the total catches by Basque fishing fleet (Ekonomiaren Garapen eta Azpiegitura Saila, 2019).

The fishing activity in the Basque Country is largely concentrated in the Ports of Bermeo, Ondarroa, Pasaia, Getaria and Hondarribia. However, most of the albacore caught by the Basque fleet is sent to the Ports of Getaria and Hondarribia (Figure 4) (Ekonomiaren Garapen eta Azpiegitura Saila, 2019).
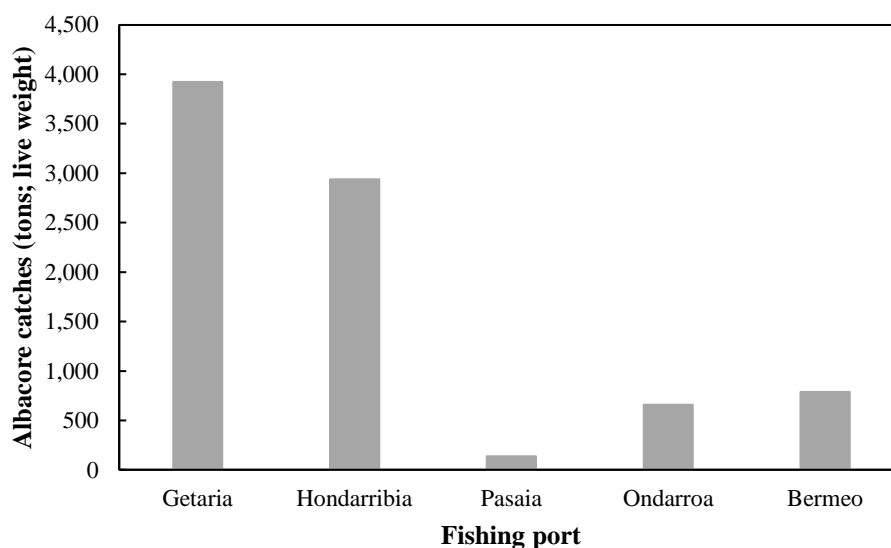
Figure 4. Albacore catches in Basque Country's Ports. Own elaboration from the statistical information on fisheries sector provided by the Department of Agriculture, Fisheries and Food of the Basque Government (Ekonomiaren Garapen eta Azpiegitura Saila, 2019).

1.1.8. Albacore with Esuko Label

Albacore that complies the requirements detailed in Table 1 can be identified with the Eusko label distinctive (Eusko Label Bereizgarria Duten Hegaluze eta Hegalaburraren Arautegi teknikoa/Reglamento Técnico de Bonito del Norte y Atún Rojo con distintivo Eusko Label, 2015). This distinctive aim to improve the quality of albacore as well as to ensure the sustainability of North Atlantic albacore stock, since albacore with Eusko label must be caught using traditional fishing methods.

Albacore with Eusko label is identified by a white seal placed in the tail that contains the Eusko Label symbol as well as a control number (Eusko Label Bereizgarria Duten Hegaluze eta Hegalaburraren Arautegi teknikoa/Reglamento Técnico de Bonito del Norte y Atún Rojo con distintivo Eusko Label, 2015).

It is intended to distinguish with the Eusko label distinctive as many individuals as possible in order to promote the fishery activity in the Basque Country.

Table 1. Requeriments of Eusko label distinctive (Eusko Label Bereizgarria Duten Hegaluze eta Hegalaburraren Arautegi teknikoa/Reglamento Técnico de Bonito del Norte y Atún Rojo con distintivo Eusko Label, 2015).

Species	*Thunnus alalunga*
Fleet	Basque fishing fleets
Fishing gear	Baitboat, trolling line. Caught one by one
Port	Hondarribia, Getaria, Bermeo, Ondarroa, Pasaia, Donostia, Lekeitio
Size	> 4 kg
Freshness	"Extra", "A"

1.1.9. Consumption data

Albacore can be marketed as fresh or refrigerated fish, as fresh or refrigerated fish for further processing and as frozen fish and as frozen fish for further processing (Secretaría General de Pesca, 2017). Nevertheless, the albacore data consumption provided by the Ministry of Agriculture, Fisheries and Food is included in the tuna and bonito consumption (Ministerio de Agricultura Pesca y Alimentación, 2019). Furthermore, the consumption of canned tuna is counted separately (Ministerio de Agricultura Pesca y Alimentación, 2019).

According to the data from the Ministry of Agriculture, Fisheries and Food (Ministerio de Agricultura Pesca y Alimentación, 2019), tuna and bonito consumption in Spain was 21,512.37 tons in 2018 (Figure 5). This represented 4.7% of total fish consumption. In the Basque Country, 1,764.51 tons were consumed in 2018, representing 8.2% of total tuna and bonito consumed in Spain (Ministerio de Agricultura Pesca y Alimentación, 2019).
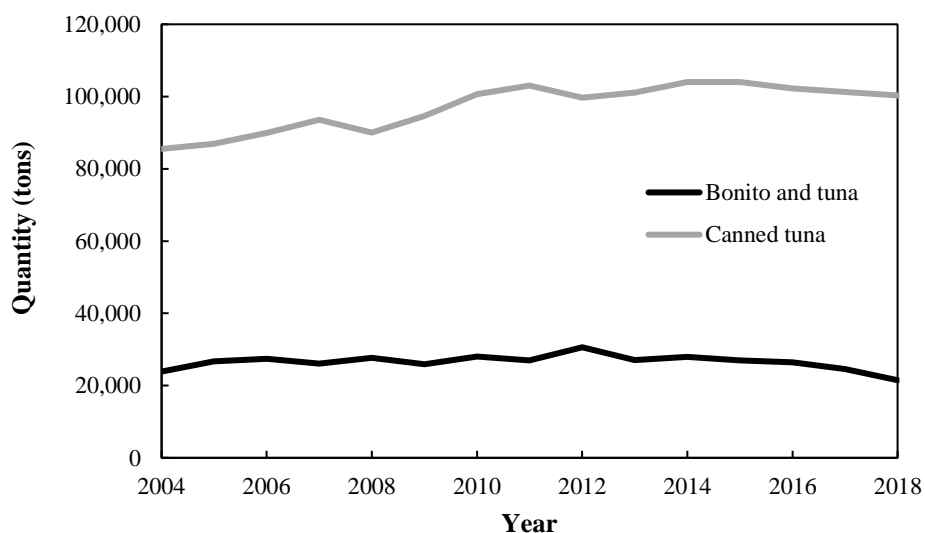
Figure 5. Consumption data of bonito and tuna (black) and canned tuna (grey) in Spain (2004−2018). Own elaboration from the Ministry of Agriculture, Fisheries and Food database of consumption (Ministerio de Agricultura Pesca y Alimentación, 2019).

Tuna and bonito consumption in Spain has remained constant over the years. However, it has been slightly decreasing since 2016 (Figures 5 and 6). By contrast, the price has increased over time (Figure 7) (Ministerio de Agricultura Pesca y Alimentación, 2019). Regarding canned tuna, its consumption in tons has increased over time, remaining almost stable since 2016 (Figure 5). However, per capita consumption has been decreasing since 2016 (Figure 6), may be due to the increase in price (Figure 7) (Ministerio de Agricultura Pesca y Alimentación, 2019).
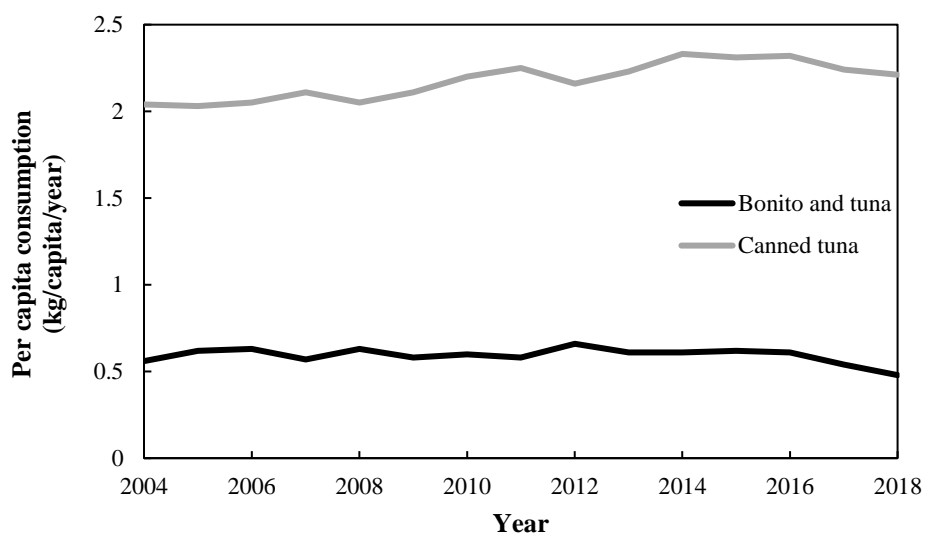
Figure 6. Per capita consumption of bonito and tuna (black) and canned tuna (grey) in Spain (2004–2018). Own elaboration from the Ministry of Agriculture, Fisheries and Food database of consumption (Ministerio de Agricultura Pesca y Alimentación, 2019).
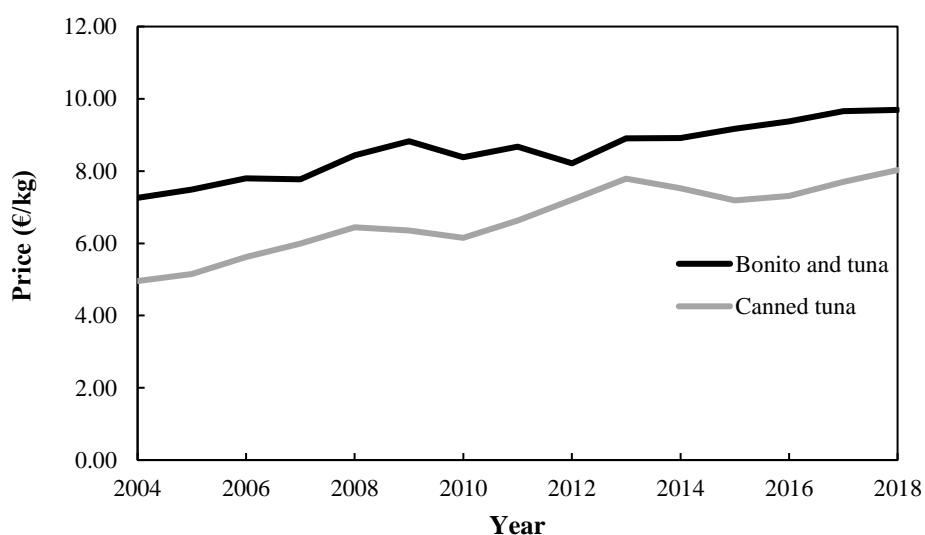
Figure 7. Price evolution of bonito and tuna (black) and canned tuna (grey) in Spain (2004–2018). Own elaboration from the Ministry of Agriculture, Fisheries and Food database of consumption (Ministerio de Agricultura Pesca y Alimentación, 2019).

In the Basque Country, the consumption of tuna and bonito has fluctuated over time (Figures 8 and 9) (Ministerio de Agricultura Pesca y Alimentación, 2019). The consumption in tons showed a slightly decreasing trend, showing the lowest level of recent years in 2018 (Figure 8). The price has increased considerably since 2004 (Figure 10). The consumption of canned tuna in the Basque Country has been increasing since 2004, although it slightly decreased in 2018 (Figures 8 and 9) (Ministerio de Agricultura Pesca y Alimentación, 2019).
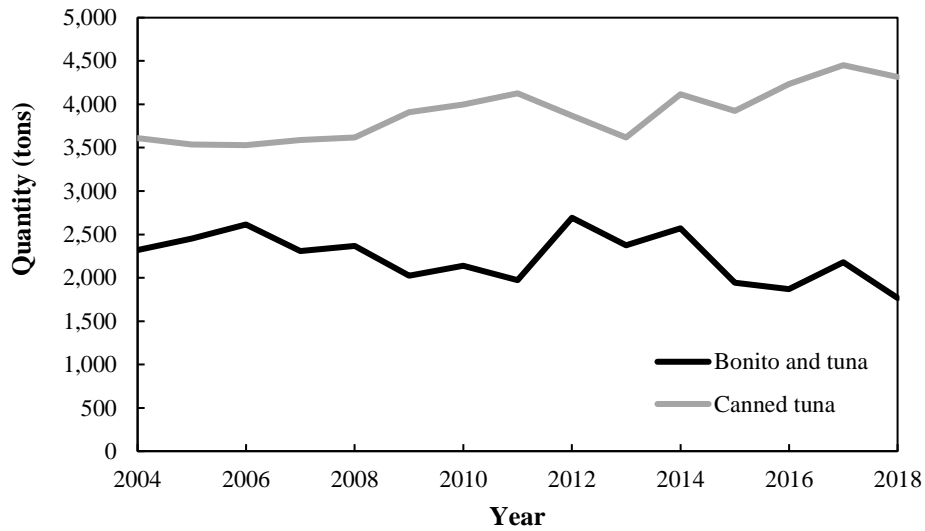
Figure 8. Consumption data of bonito and tuna (black) and canned tuna (grey) in the Basque Country (2004−2018). Own elaboration from the Ministry of Agriculture, Fisheries and Food database of consumption (Ministerio de Agricultura Pesca y Alimentación, 2019).
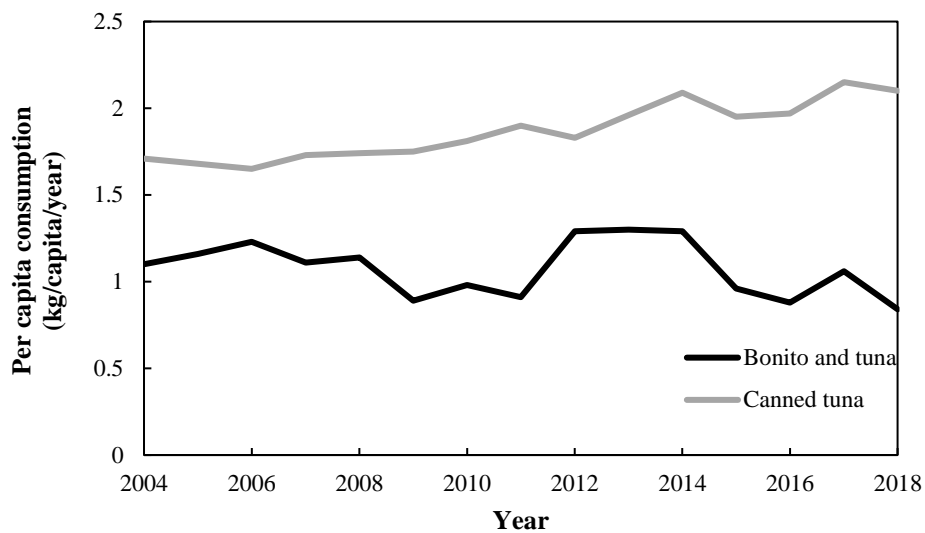
Figure 9. Per capita consumption of bonito and tuna (black) and canned tuna (grey) in the Basque Country (2004−2018). Own elaboration from consumption the Ministry of Agriculture, Fisheries and Food database of consumption (Ministerio de Agricultura Pesca y Alimentación, 2019).
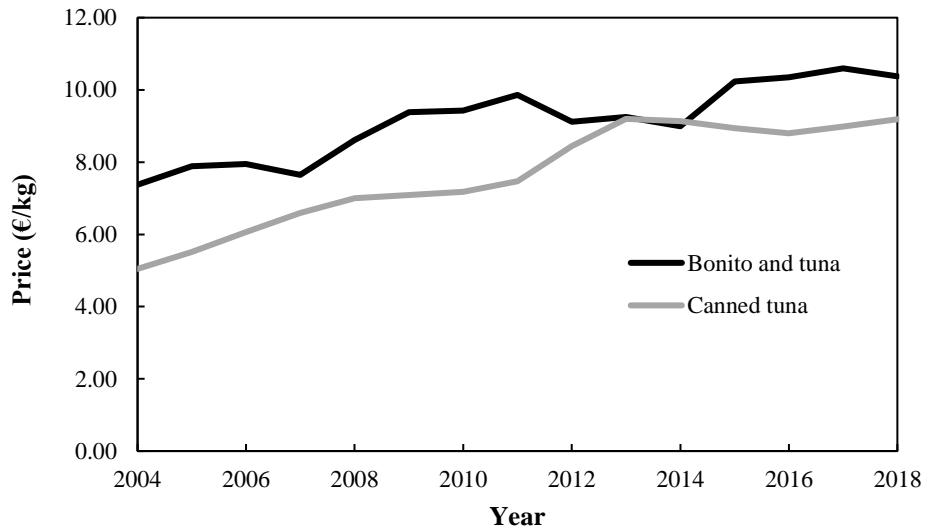
Figure 10. Price evolution of bonito and tuna (black) and canned tuna (grey) in the Basque Country (2004–2018). Own elaboration from consumption the Ministry of Agriculture, Fisheries and Food database of consumption (Ministerio de Agricultura Pesca y Alimentación, 2019).

1.2. Preservation of albacore

Since albacore is a seasonal fish species and a perishable product, it is necessary to use processing and preservation methods that ensure its availability throughout the year while retaining its initial quality. Conventional preservation technologies such as heat treatment, refrigeration or freezing have been commonly used in seafood products (Misra et al., 2017). Freezing is the best technology for the long-term preservation of fish products.

In the last decades, several innovative technologies, such as ultrasounds, magnetic-fields or high-pressures have been proposed as alternative to conventional preservation methods (Angsupanich & Ledward, 1998; Misra et al., 2017; Otero et al., 2017; Z. Shi et al., 2019). Among these technologies, high-pressures has shown the most promising results (Chéret et al., 2005; Teixeira et al., 2014; Yagiz et al., 2009).

1.2.1. Freezing and frozen storage

Freezing is the best preservation technology for ensuring albacore year-round availability, as well as extending the shelf-life of albacore. However, freezing and frozen storage lead to several quality changes in albacore quality (Q. Jiang et al., 2019).

1.2.1.1. Fundamentals

Freezing consists in lowering the temperature of a product below its freezing point, in which most of the water turns into ice (Gökoğlu & Yerlikaya, 2015c).

Freezing has two successive processes: nucleation and crystal growth. Nucleation consists in the formation of a thermodynamically stable new crystal (Dalvi-Isfahan et al., 2017). Once this crystal is formed, the crystal growth occurs by the addition of other particles. Nucleation may be homogeneous or heterogeneous. Homogeneous nucleation occurs spontaneously in pure water without impurities, whereas heterogeneous nucleation occurs when there are impurities that act as nucleating agents.

The freezing process has three stages:

- Pre-cooling: The temperature decreases up to the freezing point of the product. There is no formation of ice crystals. It involves the release of sensible heat.

- Phase transition: Water turns into ice: It involves the release of latent heat. In pure water, temperature remains constant at the freezing point until all the water is converted into ice. In food systems, the freezing point gradually decreases as the solute concentration increases during the conversion of water into ice.

- Sub-cooling: The temperature decreases below the freezing point up to the freezer temperature. It involves the release of sensible heat.

1.2.1.2. Effects of freezing and frozen storage on quality

Physical and chemical changes take place during freezing and frozen storage, including structural changes, protein denaturation and lipid oxidation.

➢ Structural changes

During freezing, water converts to ice leading to structural damages. The freezing rate determines the extent of cell damage. Slow freezing results in irregular large ice crystals located outside the cells, resulting in an increased salt concentration in the extracellular space and damage in muscle tissue, as well as an increased salt concentration in the extracellular space, promoting chemical changes in muscle tissue. (Boziaris, 2014; Gökoğlu & Yerlikaya, 2015b). Fast freezing results in small and regular ice crystal located in both intracellular and extracellular spaces, leading to lesser structural damage in muscle tissue and a lesser migration of water (Boziaris, 2014; Gökoğlu & Yerlikaya,

2015b). During thawing, extracellular ice melts and cannot be reabsorbed by cells, leading to weight loss (Boziaris, 2014; Y. Zhang & Ertbjerg, 2019).

➢ Protein denaturation

Protein denaturation and the subsequent loss of functional properties of proteins are related to undesirable changes in water holding capacity, extractability or texture (Jaczynski et al., 2012; S.-T. Jiang & Lee, 2005).

Protein denaturation can be explained by several mechanisms: Proteins are directly affected by freezing through ice crystal formation and subsequent dehydration of proteins (Jessen et al., 2014). Changes in pH as a consequence of changes in acid-base equilibrium caused by the increased concentration of solutes in the non-frozen aqueous phase also can be involved in these changes in the native structure of proteins (Jaczynski et al., 2012). In addition, the increased ionic strength of the non-frozen aqueous phase caused by the conversion of water into ice leads to changes in protein conformation, thus resulting in protein denaturation (Venugopal, 2005b).

In gadoid species, formaldehyde (FA) and dimethylamine (DMA) resulting from the degradation of trimethylamine oxide (TMAO) by the endogenous enzyme trimethylamine-oxide aldolase (TMAOase), can react with functional groups of protein side chains, leading to protein denaturation (Jaczynski et al., 2012; Sotelo et al., 1995).

Free fatty acids (FFA) resulting from hydrolysis of lipids also contribute to protein denaturation (Jaczynski et al., 2012).

Autooxidation of lipid is also related to protein denaturation due to the formation of cross-linkages between lipid oxidation products and proteins (Venugopal, 2005b).

➢ Lipid damage

Fish muscle is highly susceptible to lipid oxidation due to its high content in polyunsaturated fatty acids.

- Lipid hydrolysis: Ice crystal formation during freezing could release several endogenous enzymes. These enzymes hydrolysate triacylglycerides and form free fatty acids (FFA) during frozen storage (Gökoğlu & Yerlikaya, 2015b). Lipid hydrolysis involves texture changes by the interaction of FFA with proteins, off-

flavors formation and accelerates the development of lipid oxidation (Méndez et al., 2017).

- Lipid oxidation: Ice crystal can disrupt cells and, subsequently, release pro-oxidant substances for lipid oxidation (Benjakul & Bauer, 2001). Lipid oxidation leads to off-flavors, off-odors, undesirable changes in texture and in muscle functionality and nutritional losses (Gökoğlu & Yerlikaya, 2015b). The lipid oxidation process has three stages:

  - Initiation stage: Hydrogen atom is removed from a fatty acid in the presence of trace materials, enzymes, light or heat and results in a lipid alkyl radical (Zaritzky, 2008). In the presence of oxygen, the lipid alkyl radical reacts to form a lipid peroxyl radical (Medina-Meza et al., 2014).

  - Propagation stage: The lipid peroxyl radical subtracts a hydrogen from other fatty acid, leading to hydroperoxide molecules and other lipid alkyl radicals (Zaritzky, 2008). Hydroperoxides and peroxides are the primary lipid oxidation compounds.

  - Final stage: Hydroperoxides, which are highly unstable, break down leading to several low molecular products, such as epoxides, aldehydes or ketones (secondary lipid oxidation compounds) (Pereira de Abreu et al., 2010). These products are responsible for rancid flavor.

- Sensorial quality

All these physical and chemical changes mean several changes in fish quality parameters:

- Weight loss: Muscle cell disruption caused by ice crystal formation promotes the release of intracellular water to extracellular space (Leygonie et al., 2012). Since proteins are denatured during freezing and frozen storage, they lose their ability to bound water, which cannot be reabsorbed during thawing and it is released as exudate (Leygonie et al., 2012; Y. Zhang & Ertbjerg, 2019). Weight loss involves important economic losses, being an important issue for fish industry (Truong et al., 2016). Additionally, it affects the appearance, texture and nutritional quality of fish (loss of water-soluble proteins, minerals and vitamins) (Gökoğlu & Yerlikaya, 2015e).

- Texture changes: It is associated with lipid-protein interaction, protein denaturation and aggregation, and cell damage caused by ice crystal formation (Jaczynski et al., 2012; Martinez et al., 2010). Weight loss is correlated with texture changes, resulting in a dry and stringy texture (Kolbe & Kramer, 2007).

- Color changes: Discoloration by oxidation of myoglobin to metmyoglobin (Venugopal, 2005b).

- Flavor changes: It consists on the development of rancid flavors due to lipid oxidation (Venugopal, 2005b).

Several innovative technologies have been proposed for reducing the quality loss caused by the freeze-thaw process and the frozen storage on seafood products, such as the application of high-pressures (Chevalier et al., 2000b), ultrasounds (Antunes-Rohling et al., 2021; Q. Sun et al., 2019) or magnetic fields (Otero et al., 2017). Despite their promising results, these technologies have not been implemented industrially due to their high cost (James et al., 2015). Recently, high-pressure pretreatments before freezing have been proposed for improving the quality of frozen seafood products (Aubourg, Torres, et al., 2013; Torres et al., 2014; Truong et al., 2016; Vázquez et al., 2013, 2018) showing promising results, such as the inhibition of the formation of FA and DMA (Vázquez et al., 2018), the inhibition of the development of lipid damage (Vázquez et al., 2013) or the enhancement of the sensorial quality (Torres et al., 2014).

1.2.2. High-pressure Processing (HPP)

Consumers demand safe and minimally processed products, as well as with an extended shelf life. In this regard, recent research has focused on non-thermal technologies. One of the most promising technologies is high-pressure processing (HPP). High-pressure processing (HPP) can be employed as an alternative to conventional treatments (e.g., heat treatment) because reduces undesirable changes in sensorial and nutritional properties inherent to these treatments while enlarges shelf life. Furthermore, HPP is a clean technology, since it generates little waste and it spends less energy than heat treatments (Priyadarshini et al., 2019).

The effectiveness of HPP treatment depends on three parameters: pressure level, pressurization time and temperature. Optimization of these parameters is a key factor for improving the quality of seafood products (Barba et al., 2015b; Ghafoor et al., 2020).

Furthermore, several intrinsic parameters of the food matrix can affect its effectiveness, such as pH, composition or water activity (Pérez-Andrés et al., 2018).

Initial research on HPP treatment dates from 1899 and aimed to preserve milk and other food products (Alves de Oliveira et al., 2017; Ghafoor et al., 2020). Food products subjected to HPP treatment were firstly commercialized in Japan in 1993 (Tao et al., 2014). In the last decade, HPP has been applied in different food products, such as vegetables, meat, seafood or juice and beverages (Heinz & Buckow, 2010; Misra et al., 2017).

1.2.2.1. Fundamentals

The HPP treatment process is described as follows (Figure 11): Product is placed in a pressure vessel (1). The vessel is filled with a pressure-transmitting medium, usually water (2). Pressure then increases in the vessel by a hydraulic pump or by using pistons that reduces the volume of the chamber (3). Then, the pressure is kept constant for a designated time. Finally, the system is depressurized (4) (Barba et al., 2018; Ghafoor et al., 2020; Heinz & Buckow, 2010; Tao et al., 2014).

Hence, the pressure cycle has three stages (Ghafoor et al., 2020; Tao et al., 2014):

- Pressurization: Pressure increases until the desired pressure is reached.

- Pressure holding: The pressure is maintained without additional energy input.

- Decompression: Once elapsed the pressure holding time, the pressure is released.

HPP is governed by the Le Châtelier's principle and the Isostatic principle, which determine the effect of HPP on food products. In accordance to the Le Châtelier's principle, all reactions that involve a volume reduction are favored, whereas reactions that involve a volume increase are inhibited (Bajovic et al., 2012; Puértolas & Lavilla, 2020). As a consequence, macromolecules such as proteins or enzymes can change their native structure, whereas low molecular weight components such as vitamins, minerals or flavor compounds, which are responsible for the nutritional and sensorial quality, are not affected by pressure (Barba et al., 2015b; Campus, 2011). In accordance to the Isostatic principle, the pressure is transmitted uniformly and instantaneously to the product. Thus, HPP is independent of the size, shape and volume of the food product (Barba et al., 2018).
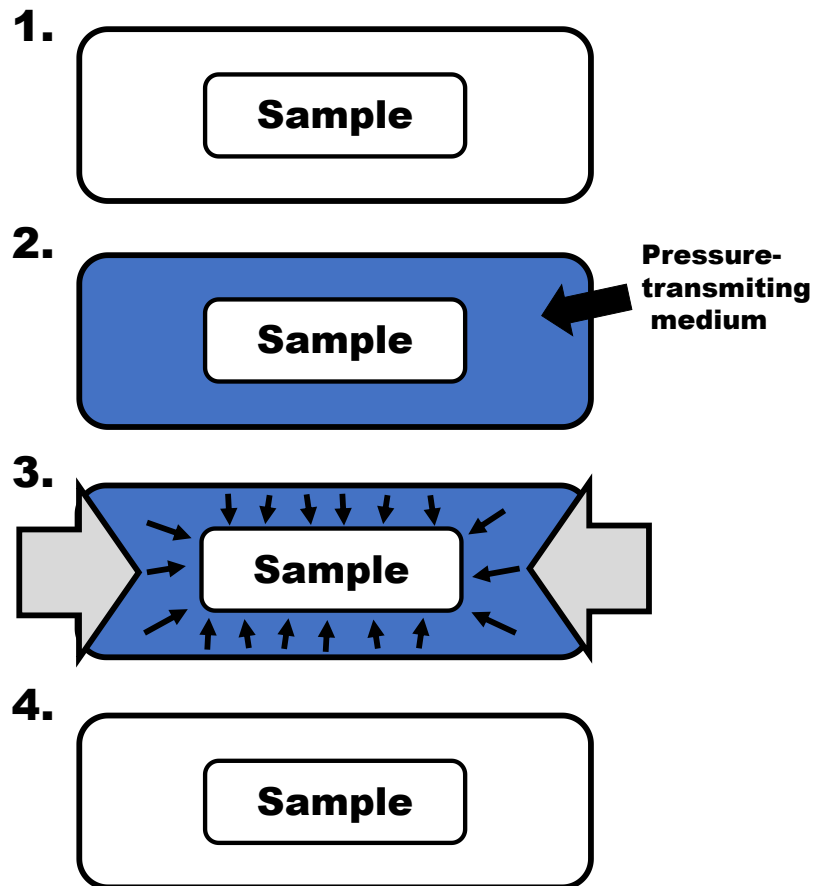
Figure 11. Operation of a HHP unit. Adapted from Hiperbaric.

Although HPP is a non-thermal technology, the temperature of the food product increases (adiabatic heat) due to the compression. In the case of pure water, the temperature increases around 3 ºC /100 MPa, whereas in samples with a high content in fat it increases around 9 ºC/100 MPa (Barba et al., 2018; Tao et al., 2014). The temperature decreases after decompression, reaching slightly lower values than before HPP treatment (Ghafoor et al., 2020; Tao et al., 2014).

1.2.2.2. Effects of HPP treatment on fish quality

Pressure induces some changes in proteins and lipids of muscle tissues that lead to several changes in the fish quality.

➤ Changes in proteins

Changes in proteins induced by pressure are key to explain the effects of HPP on color, texture or water holding capacity of fish muscle (Zhu et al., 2008).

In accordance with the Le Châtelier's principle, hydrophobic and electrostatic interactions are affected by pressure, while covalent bonds are intact because of its low

compressibility (Alves de Oliveira et al., 2017; Ghafoor et al., 2020). Hence, tertiary and quaternary structures (mainly maintained by hydrophobic and electrostatic interactions) are sensitive to pressure, whereas the primary structure of proteins (maintained by covalent bonds) is more stable to pressure (Truong et al., 2015). Hydrogen and disulfide bonds are rarely affected by pressure (Angsupanich & Ledward, 1998) and even new hydrogen bonds can be formed (Chevalier et al., 2001). All these changes in protein conformation can result in unfolding, denaturation, aggregation, precipitation or gelation (Alves de Oliveira et al., 2017; Bajovic et al., 2012). Below 300 MPa, the pressure only affected the tertiary and quaternary structures of the proteins, causing reversible denaturation of proteins (Rastogi et al., 2007). Above 300 MPa, pressure can also affect the secondary structure, causing irreversible denaturation (Pérez-Andrés et al., 2018).

Myofibrillar proteins have been reported to be more sensitive to pressure than sarcoplasmic proteins (Angsupanich & Ledward, 1998; Christensen et al., 2017). Collagen seems not to be affected by pressure (Truong et al., 2015). Previous research showed that myosin denaturation occurred at 100–200 MPa in several fish species whereas actin denaturation occurred around 200 MPa (Angsupanich & Ledward, 1998; Chevalier et al., 2000b; Christensen et al., 2017; Rastogi et al., 2007). Sarcoplasmic proteins were denatured around 300–400 MPa (Alves de Oliveira et al., 2017; Angsupanich & Ledward, 1998).

HPP can also induce protein oxidation throughout the interaction of free radicals with proteins, resulting in changes in color and texture of fish muscle (Pérez-Andrés et al., 2018). Protein oxidation could be correlated with lipid oxidation, since they have similar routes (Guyon et al., 2016). Carbonyl group content, which indicates the extent of protein oxidation, was increased after HPP treatment at 300 MPa for 10 min when compared to the control (Ojagh et al., 2011).

➤ Changes in lipids

Impact of HPP on lipid damage depends on HPP treatment conditions (pressure, time and temperature), fish species, handling, storage conditions and type of muscle (white or red muscle) (Aubourg, 2018; Truong et al., 2015).

No effect of HPP on lipid content was observed in several fish species, such as Chilean jack mackerel (*Trachurus murphyi*) or Coho salmon (*Oncorhynchus kisutch*) (Maluenda et al., 2013; Ortea et al., 2010).

Research on the effect of HPP on the free fatty acid profile of seafood products is scarce and results are controversial. Some authors have reported an increase of saturated fatty acids and a decrease of the unsaturated ones in several seafood samples after HPP treatment (de Alba et al., 2019; Gómez-Estaca et al., 2016; Shang et al., 2014). These changes might be related to the cell membrane disruption induced by pressure and the subsequent changes in lipid membranes (de Alba et al., 2019). By contrast, other authors did not report any effect of HPP treatment on free fatty acid profile (Yagiz et al., 2009).

Lipid oxidation is promoted by HPP through several mechanisms. HPP treatment leads to the release of metal ions from the prosthetic group, which catalyze lipid oxidation. In addition, denaturation of hemeproteins such as myoglobin induced by pressure involves a great availability of catalytic heme group, promoting lipid oxidation. HPP also induces the disruption of cellular membranes and subsequently involves the exposure of unsaturated lipids to enzymes and free metal radicals (Alves de Oliveira et al., 2017; Medina-Meza et al., 2014).

TBARS (Thiobarbituric acid reactive substances) index is commonly used for measuring the extent of lipid oxidation in seafood products. In general, increased TBARS values have been reported in several fish species immediately after HPP treatment (Angsupanich & Ledward, 1998; Chevalier et al., 2001; Jiranuntakul et al., 2018; Teixeira et al., 2014; Yagiz et al., 2009). By contrast, no changes in TBARS value have been found after applying HPP treatment in hilsa (*Tenualosa ilisha*) (250 and 350 MPa for 10 min) (Chouhan et al., 2015) or mackerel (*Scomber* spp.) (100, 300 and 500 MPa for 2 or 5 min) (de Alba et al., 2019). However, when storage is considered, lipid oxidation is reported to be inhibited in HPP-treated samples compared to the controls (Chouhan et al., 2015; Méndez et al., 2017; Ramirez-Suarez & Morrissey, 2006; Yagiz et al., 2009).

Regarding the free fatty acid (FFA) formation, which indicates the extent of lipid hydrolysis, no significant changes after HPP treatment were found in turbot (*Scophthalmus maximus*) (100–200 MPa for 15 and 30 min) (Chevalier et al., 2001), whereas a higher FFA formation was observed in HPP-treated Coho salmon (170 MPa for 30 s) and hilsa (350 MPa for 10 min) than in control samples immediately after treatment (Chouhan et al., 2015; Ortea et al., 2010). As a result of lipid hydrolysis development, texture degradation, lipid oxidation promotion and off-odors development (Truong et al., 2015) may occur in fish products. Lipid hydrolysis is mainly caused by

endogenous enzymes such as lipases or phospholipases (Aubourg, 2018; Ortea et al., 2010). However, there are few research works about the impact of HPP on enzymes responsible for lipid hydrolysis.

> ➢ Sensorial quality
>> • Color

It has been widely reported that HPP involves adverse changes in fish muscle characterized by a loss of translucency and an increased whiteness, reaching an appearance similar to cooked fillets (Chouhan et al., 2015; Suemitsu & Cristianini, 2019). These changes are related to the denaturation of sarcoplasmic and myofibrillar proteins (Alves de Oliveira et al., 2017). Myoglobin is the major pigment on tuna meat and gives it its characteristic red color. Hence, several myoglobin alterations, such as heme displacement or release, iron release or ferrous myoglobin oxidation to ferric metmyoglobin (Alves de Oliveira et al., 2017; Jiranuntakul et al., 2018; Ramirez-Suarez & Morrissey, 2006) can result in a color deterioration after HPP treatments. Although it has been reported that HPP could not directly induce myoglobin oxidation, lipid and protein oxidation may promote it (Alves de Oliveira et al., 2017).

HPP treatment of fish muscle generally leads to an increase in L^* value (lightness) (Chéret et al., 2005; de Alba et al., 2019; Jiranuntakul et al., 2018; Ramirez-Suarez & Morrissey, 2006; Senturk & Alpas, 2013; Yagiz et al., 2009). The effect is strongly dependent on treatment conditions (Jiranuntakul et al., 2018; Ramirez-Suarez & Morrissey, 2006; Suemitsu & Cristianini, 2019).

The effect of HPP on a^* value (redness) depends on fish species and treatment conditions (Gómez-Estaca et al., 2009). Whereas increased in skipjack tuna (*Katsuwonus pelamis*) after HPP treatments at 150 to 600 MPa for 1, 3 and 5 min with respect to the non-treated samples (Jiranuntakul et al., 2018), a decreasing trend was reported in HPP-treated mackerel (100, 300 and 500 MPa for 2 and 5 min) (de Alba et al., 2019).

The b^* value (yellowness) also increases due to the pressure (Jiranuntakul et al., 2018; Ramirez-Suarez & Morrissey, 2006; Suemitsu & Cristianini, 2019). This increase has been related to lipid oxidation (Aubourg, Rodríguez, et al., 2013).

- Texture

Changes in texture are associated with changes in proteins induced by pressure, such as unfolding, denaturation, aggregation or gelation (Chéret et al., 2005; Rastogi et al., 2007). HPP treatment induces muscle compaction, characterized by a reduction of the extracellular space (Chéret et al., 2005) and can affect texture due to an increase in protein-protein interactions and bond formation (Truong et al., 2015). Furthermore, texture is highly related to water holding capacity (Kolbe & Kramer, 2007). The effect of HPP treatment on texture of fish muscle is variable according to treatment conditions (pressure level and pressure time), composition and methodology (de Alba et al., 2019). In this regard, texture profile analysis (TPA) is one of the most commonly used methods for texture analysis. It measures the hardness, adhesiveness, cohesiveness, springiness and chewiness of samples from a force-deformation curve (Chéret et al., 2005).

The hardness generally increased after HPP treatment (Angsupanich & Ledward, 1998; Chéret et al., 2005; Chouhan et al., 2015; de Alba et al., 2019; Ramirez-Suarez & Morrissey, 2006; Yagiz et al., 2009). This hardness increase was higher as the pressure level increased (Chouhan et al., 2015; Yagiz et al., 2009) and has been attributed to the unfolding of actin and sarcoplasmic proteins and formation of new hydrogen-bonded networks (Angsupanich & Ledward, 1998).

Higher springiness (Chouhan et al., 2015; Jiranuntakul et al., 2018; Yagiz et al., 2007) and chewiness (Angsupanich & Ledward, 1998; Chouhan et al., 2015; Jiranuntakul et al., 2018; Yagiz et al., 2007, 2009) values than in the control were also reported in several fish species after HPP treatment.

The effect of HPP treatment on cohesiveness is less consistent. While Chouhan et al. (2015) observed an increase in cohesiveness after HPP treatment at 350 MPa for 10 min in hilsa fillets, de Alba et al. (2019) did not find differences as a result of HPP treatments (100–500 MPa for 2–5 min) in mackerel samples. Similarly, there is not a clear trend about the impact of HPP treatment on adhesiveness, since no effect was found in HPP-treated Atlantic salmon (*Salmo salar*) (150 and 500 MPa for 15 min) (Yagiz et al., 2009).

- Water retention

HPP treatment (200–400 MPa for 1–10 min) decreased the weight loss of razor clam with respect to the controls (Xuan et al., 2018). The same effect was observed in other muscle foods (Grossi et al., 2014; Souza et al., 2011). This fact may be caused by the muscle

compaction induced by the HPP treatment, which involves an increased intra- and intermolecular bond formation and protein gelation (Jiranuntakul et al., 2018; Rastogi et al., 2007) and leads to better water retention. Several authors have reported that HPP treatment allowed to minimize the weight loss increase inherent to storage (Chéret et al., 2005; Hurtado et al., 2000).

Research on the impact of HPP treatment on cooking loss of fish products is scarce. Shang et al. (2015) observed that lower HPP treatments (100–300 MPa for 10 min) resulted in a lower cooking loss than the controls. However, this positive effect of HPP was lost when higher treatments were applied (300–600 MPa for 10 min) (Shang et al., 2015).

There is not a clear effect of HPP treatment on water holding capacity (WHC). WHC decrease after HPP treatment was found in several seafood products, such as cod (*Gadus morhua*), Atlantic salmon and albacore *carpaccios*, Atlantic mackerel or Atlantic salmon (Christensen et al., 2017; Gómez-Estaca et al., 2009; Lakshmanan et al., 2007), while no changes were reported in sea bass (*Dicentrarchus labrax* L.) (Chéret et al., 2005).

Hence, water retention of muscle tissues may be improved by the application of HPP treatments (Chéret et al., 2005). As commented before, weight loss is an important issue in frozen products due to its economic implications. Thus, in this Thesis, high-pressure was proposed to decrease the weight losses of frozen albacore inherent to the freeze-thaw process.

2.HYPOTHESIS AND AIMS

Freezing is widely used for long-term preservation of seafood products. However, the freeze-thaw process and the frozen storage involves several physical and biochemical changes that affect the quality of these products. In this sense, weight loss is one of the most important issues of frozen products because it leads to economic losses for fish industry, as well as the loss of some proteins and vitamins. Hence, weight loss reduction is an important concern of the seafood industry.

High-pressure processing may be a suitable technology for improving the water retention of muscle proteins of several seafood products. Therefore, its application as a pretreatment in frozen product could decrease the weight losses caused by the freeze-thaw process and the frozen storage.

The hypothesis of this Thesis was: The application of high-pressure pretreatments is a useful strategy to improve the fish quality (e.g., weight losses). The general aim to demonstrate this hypothesis was to improve the quality of frozen albacore through the application of high-pressure pretreatments, especially for reducing weight losses.

To achieve this general aim, several partial aims were established:

1. To identify the fish quality changes caused by HPP.

2. To identify the main causes of quality loss due to the freezing process and the frozen storage in albacore.

3. To determinate the best HPP treatments for reducing weight losses in albacore.

4. To determinate the best HPP treatments for decreasing weight losses of albacore while retaining fresh-like quality.

5. To study the stability of albacore during long-term storage after the application of HPP processing.

6. To improve the quality of albacore during long-term storage in order to ensure its availability throughout the year.

7. To determinate the impact of different freezing conditions on albacore subjected to HPP treatments before freezing.

8. To study the potential benefits of applying HPP pretreatments in frozen albacore.

3.MATERIAL AND METHODS

The material and methods used in the present thesis are described below. The specific material and methods of each chapter will be detailed in its corresponding section.

3.1. Samples

Albacore was caught in the Bay of Biscay (subarea 28.8). The samples were purchased from a local supplier (Pescados Marisa, Portugalete, Spain), where they were beheaded, gutted and filleted (Figure 12). In order to reduce the influence of external factors, all the albacore individuals came from the same fish shoal and boat, and they were landed at the port on the same day.

Figure 12. Preparation of raw material.

The albacore steaks (Figure 13) were immediately transported to the pilot plant for processing.

Steaks from different fishes were randomly distributed into the different batches. Steaks with irregular shape or an unusual size were discarded to ensure the homogeneity of the samples.

The albacore steaks were vacuum packaged individually in polyethylene bags at 80 mbars. After the packaging process, samples were stored at refrigeration (4 ± 2 ºC).
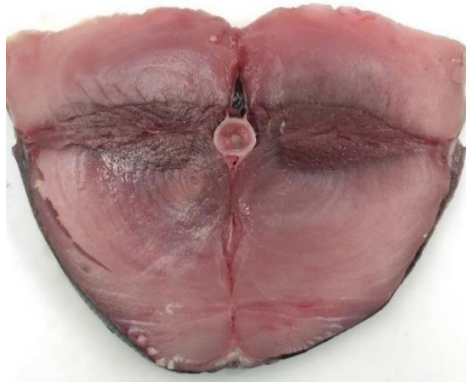
Figure 13. Albacore steak.

3.2. Processing

3.2.1. High-pressure Processing (HPP)

HPP treatments were applied in a 55–L high-pressure unit (WAVE 6000/55HT; Hiperbaric, Burgos, Spain) (Figure 14) using water as the pressuring medium. Inlet water temperature was 6 ºC. Albacore steaks were subjected to HPP treatments once individually packaged (Figure 15).

Figure 14. High-pressure unit.

Figure 15. High-pressure processing of albacore steaks.

3.2.2. Freezing, frozen storage and thawing

Freezing was carried out in an air blast-freezer (CAS-30B, ABI Co. Ltd., Chiba, Japan). The freezing chamber has 10 extractable trays, where the albacore batches were equally distributed. Air temperature and air velocity were set at –20 ºC and 5 m s^{-1}, respectively. In the Study 5, different freezing conditions were also considered. Freezing was concluded when the geometrical center of albacore steaks reached –20 ºC.

Samples were stored in a freezing chamber (20 ± 2 ºC) until thawing.

Thawing was carried out in a refrigeration chamber (4 ± 2ºC) for 24 h. During the thawing process, the samples were distributed at the same height (similar temperature) on several shelves of the same refrigerated chamber, separated 10 cm from each other to facilitate the heat exchange (randomized order).

3.2.3. Cooking

Albacore steaks were wrapped individually in aluminum foil and then cooked using an oven (UFE 500, MEMMERT GmbH + Co. KG, Schwabach, Germany), at 150 °C for 30 min (Figure 16). After cooking, they were cooled at room temperature for 30 min and then individually packaged in polyethylene bags.

Figure 16. Cooked albacore steak.

Albacore cubes (2 cm × 2 cm × 2 cm) were wrapped in fours in aluminum foil and then cooked by oven (UFE 500, MEMMERT GmbH + Co. KG, Schwabach, Germany) at 105 °C for 15 min. Once cooked, they cubes were cooled at room temperature for 15 min.

3.3. Analytical methods

3.3.1. Freezing curves

During the freezing process, the temperature of albacore steaks was monitored by fiber optic temperature probes located in the geometrical center of the samples (T1, Neoptix, Quebec, Canada) connected to a data logger (ReFlex 4, Neoptix) (acquisition every 2 seconds). The albacore steaks used for the temperature control were not employed for ulterior analysis.

3.3.1.1. Freezing curve parameters

- Precooling stage: Time required for decreasing the temperature of the sample from 4 to –1 ℃ (M. Zhang et al., 2018).
- Phase transition stage: Time required for decreasing the temperature of the sample from –1 to –5 ℃ (M. Zhang et al., 2018).
- Subcooling stage: Time required for decreasing the temperature of the sample from –5 to –20 ℃ (M. Zhang et al., 2018).

- Characteristic freezing time: Time required for decreasing the temperature of the sample from –1 to –7 ℃ the temperature from –5 to –20 ℃. It indicates approximately the time in which the 80 % of water is frozen (H. W. Kim et al., 2017).

- Total freezing time: Time required for reducing the temperature from 4 to –20 ℃. It is important mainly for industrial purposes since it is the duration of the whole freezing process (Cartagena et al., 2020c).

- Freezing rate: Difference between –1 and –7 ℃ divided by the characteristic freezing time (Y. H. B. Kim et al., 2015).

3.3.2. Weight losses

3.3.2.1. Thawing loss

Thawing loss refers to the liquid losses attributed to the freeze and thawing steps. Excess drip from the steak surface was removed using filter paper. Before weighing, the surface of albacore steaks was dried with filter paper.

It was calculated as follows:

Thawing loss (%) $= \frac{(W_1 - W_2)}{W_1} \times 100$

where W_1 was the masse (g) of albacore steaks before processing and W_2 was the masse (g) of albacore steaks once thawed (24 h at 4 ℃).

In fresh (non-frozen) samples, weight loss was also calculated. It indicates the liquid lost only under gravity (without external forces) during 24 h of storage at 4 ℃ (Huff-Lonergan, 2009; Otero et al., 2017). This liquid is released from the extracellular space (Honikel & Hamm, 1994). In this case, W_1 and W_2 correspond to the masses (g) of albacore steaks before and 24 h after processing, respectively.

3.3.2.2. Cooking loss

Cooking loss indicates de liquid losses attributed to the cooking process, which is released from both extra- and intracellular spaces of the muscle tissue (Honikel & Hamm, 1994). It was determined in albacore cubes (2 cm × 2 cm × 2 cm). These cubes were weighed (W_3) and then cooked at 105 ℃ for 15 min in an oven (UFE 500, MEMMERT GmbH + Co. KG, Schwabach, Germany). Once cooked, the cubes were cooled at room temperature for 15 min and then weighed again (W_4). Before weighing, the surface of albacore steaks was dried with filter paper. Cooking loss was calculated using the following equation:

Cooking loss (%) $= \frac{(W_3 - W_4)}{W_3} \times 100$

3.3.2.3. Total weight loss

Total weight loss indicates the liquid loss caused by both freeze-thaw process and cooking. It was calculated using the following equation:

$$\text{Total weight loss (\%)} = 100 - \left[\frac{W_4}{\left(\frac{W_3}{1 - Thawing\ loss\ (\%)\ \times 0.01} \right)} \right]$$

where W_3 and W_4 were the masses (g) of albacore steaks before and after cooking, respectively. Thawing loss was expressed as a percentage.

3.3.2.4. Water holding capacity

Water holding capacity (WHC) measures the ability of meat to retain water under external forces, such as pressure, grinding or centrifugation (Honikel & Hamm, 1994; Huff-Lonergan, 2009). This liquid is released from both intra- and extracellular spaces (Honikel & Hamm, 1994). In this Thesis WHC was measured by using centrifuge forces.

Two filter papers (Whatman 1, 90 mm diameter) were folded into a cone and were introduced into a centrifuge tube (50 mL). Then, 1 ± 0.01 g of albacore muscle were weighed and placed into centrifuge tubes, over the filter papers. The centrifuge tubes were centrifugated at 2000 g for 10 min at 10 ºC (Sorvall Lengend XTR Centrifuge, Thermo Scientific, Waltham, Massachusetts, USA). Immediately after centrifugation, muscle sample was removed and the wet filter papers were weighed. Then the filter papers were dried at 105 ºC for 48 h (Sorvall Lengend XTR Centrifuge, Thermo Scientific, Waltham, Massachusetts, USA) and weighed again. WHC was expressed as the percent of bound water per 100 g of total water of the sample before centrifugation (Otero et al., 2017) according to:

$$WHC\ (\%) = \frac{\left[(M \times W_s) - (W_{p1} - W_{p2}) \right]}{(M \times W_S)} \times 100$$

where M is the moisture content of the sample expressed as a decimal ratio, W_s the mass (g) of the sample, and W_{p1} and W_{p2} the masses (g) of the filter paper before and after drying respectively.

For determination of the moisture content, the samples were weighed before and after drying at 105 °C until constant weight.

3.3.3. Color

The superficial color was evaluated using a colorimeter (Konica Minolta C–400, Tokyo, Japan) according to CIELab system. The CIELab system is a three-dimensional model that uses the coordinates $L*$ (lightness, from black (0) to white (100)), $a*$ (coordinate red/green, $+a*$ for red, $-a*$ for green) and $b*$ (coordinate yellow/blue, $+b*$ for yellow, $-b*$ for blue) to define any color (Joint ISO/CIE Standard, 2008). From these values, the total color difference (ΔE) was also calculated as follows:

$$\text{Total color difference (ΔE): } \Delta E = (\Delta L*^2 + \Delta a*^2 + \Delta b*^2)^{0.5}$$

Where $\Delta L*$, $\Delta a*$ and $\Delta b*$ correspond to differences between $L*$, $a*$ and $b*$ values of HPP-treated samples and control (non-treated) samples for each batch.

The colorimeter was standardized using a white calibration plate (C: Y=94,0, x=0.3130, y=0.3191). Three albacore steaks for each batch were measured in four different locations for each steak. The mean values of these results were calculated.

Color was measured in white and red muscle.

3.3.4. Texture profile analysis (TPA)

Texture profile analysis is an imitative test that consists on compressing a sample twice to simulate chewing (Figure 17).

The texture of albacore steaks was evaluated at room temperature using a TA.HDplus texturometer (Stable Micro Systems Ld., Goldaming, United Kingdom) equipped with 5 kg load cell capacity and a 75 mm flat-probe (P/75). At least 4 cubes of $2 \times 2 \times 2$ cm were cut from each albacore steak and 3 steaks for batch were analyzed. These cubes were compressed twice to 30% of their original height (Martinez et al., 2007). There were 5 s between the
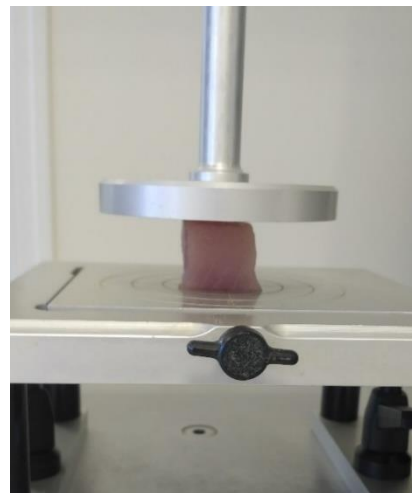
Figure 17. Texture profile analysis (TPA) of an albacore cube.

two compression cycles (Martinez et al., 2007). The crosshead pre-test, test and post-test speeds were 1-, 5- and 10-mm s^{-1}, respectively.

Several parameters were obtained from the force–time curve (Bourne, 1978), which were calculated using Texture Exponent 32 Software (Stable Micro Systems Ld., Goldaming, United Kingdom).

- Hardness (N): Maximum force (peak) of the first compression cycle. It indicates the force required for reaching a pre-determined deformation of the sample (Bourne, 1978; Kruk et al., 2011).

- Adhesiveness (N s): It is the area under the baseline between the two compression cycles. It indicates the work necessary to pull the compressing plunger away from the sample (Bourne, 1978; Kruk et al., 2011).

- Springiness (% of the initial height of the sample): Length of the second compression cycle/ length of the first compression cycle. It indicates the ability of the sample to recover its original height after the first compressing force was removed (Bourne, 1978; Martinez et al., 2004).

- Chewiness: It is the product of hardness × cohesiveness × springiness and indicates the work necessary to chew a solid sample for swallowing (Bourne, 1978; Martinez et al., 2004).

3.3.5. Thiobarbituric acid index

The extent of lipid oxidation was measured using the thiobarbituric acid reactive substances index (TBARS) using a modified method of (Vyncke, 1970). This index is based on the reaction between the malondialdehyde (MDA) contained in the trichloroacetic acid extract of the fish muscle, which is a secondary lipid oxidation product, and thiobarbituric acid (TBA) (Guyon et al., 2016; Martin-Rubio et al., 2020). This reaction results in the formation of a pink compound that can be spectrophotometrically measured. 5 g of muscle were homogenized with 10 mL of TCA extracting solution (7.5% trichloroacetic acid in water, 0.1% propyl gallate and 0.1% EDTA). The homogenate was centrifugated at 10.000 rpm for 10 min at 4 ºC. The resultant supernatant was mixed (1:1 v/v) with 0.02 M TBA in an Eppendorf tube, heated in boiling water at 95 ºC for 40 minutes and immediately cooled with running water. The absorbance was measured at 532 nm using a mixture of TBA reagent and water was (1:1

v/v) used as blank. The TBARS content was calculated from a standard curve using 1,1,3,3-tetraethoxypropane (TEP) (Truong et al., 2016) (Figure 18). The results were expressed as mg of malondialdehyde per kg of muscle (Méndez et al., 2017)
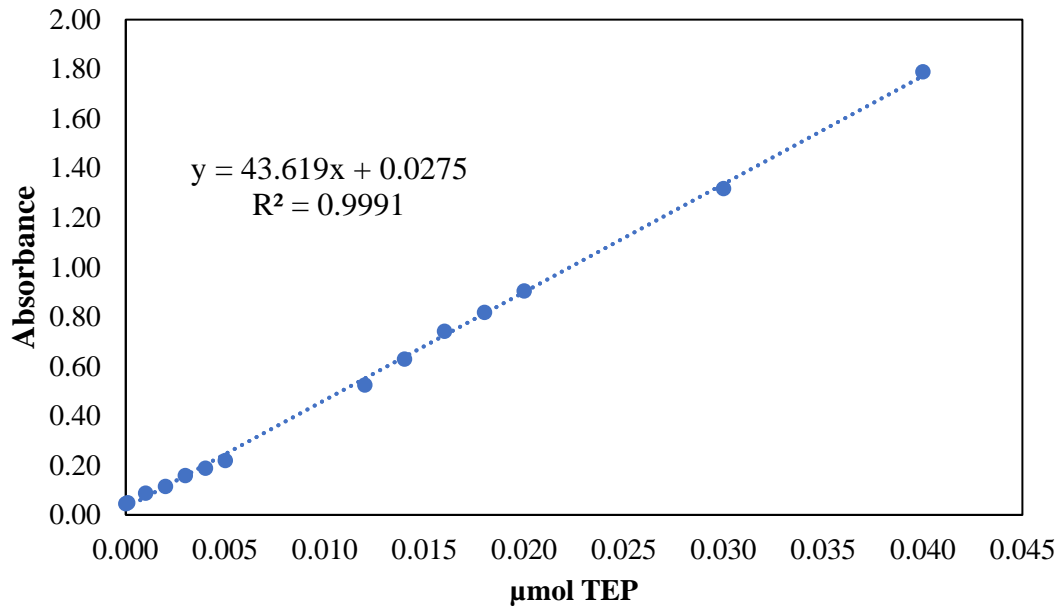
Figure 18. Calibration curve of 1,1,3,3-tetraethoxypropane (TEP).

The TBARS index was calculated as follows:

$$\text{TBARS index (mg MDA/kg)} = \frac{m_{MDA} \times C(V_{TCA} + (m_s \times M)}{m_s \times V_{aliq}}$$

Where m_{MDA} is the molecular mass of malondialdehyde (72 g); C the µmols of MDA; V_{TCA} de volume of TCA (mL); m_s the mass of the sample (g); M the moisture content of the sample (decimal ratio) and V_{aliq} the aliquot volume (mL).

3.3.6. Salt-soluble protein content

Changes in salt-soluble protein content indicate changes in protein structure caused by denaturation or aggregation of mainly myofibrillar proteins, since these proteins can be extracted in a high ionic strength buffer (ionic strength > 0.3) (Rustad, 2009). These structural changes in myofibrillar proteins may involve textural changes and a decrease of the water retention capacity (Kolbe & Kramer, 2007; Martinez et al., 2010).

2 g of albacore muscle were homogenized with 20 mL KCl solution buffer (0.6 M L^{-1} KCl, 0.05 M L^{-1} sodium phosphate, pH = 7.4) at 10,000 rpm for 30 s. The homogenate was incubated overnight at 4 ºC and. Then, the extract was centrifuged at 10,000 rpm for

10 min at 4 ºC (Sorvall Lengend XTR Centrifuge, Thermo Scientific, Waltham, Massachusetts, USA) and the supernatant was recovered. Salt-soluble protein content was determined by the Bradford method, based on the color change of Coomassie Blue dye from brown to blue in the presence of a protein solution (Bradford, 1976). The supernatant was diluted in distilled water (1:10 and 1:20). The Bradford reagent (Coomassie Blue G-250 dye) was loaded into a 96-well plate (200 µL per well) and 10 µL of aliquot were added to each well. In the case of blanks, 10 µL of distilled water were added to the Bradford reagent. The 96-well plate was incubated at room temperature for 15 min and the absorbance was measured at 595 nm using bovine serum albumin as standard (Figure 19). The results were expressed as mg of salt soluble protein per g of sample.

$$y = 2.7212x^2 - 1.1248x + 0.0929$$
$$R^2 = 0.9943$$
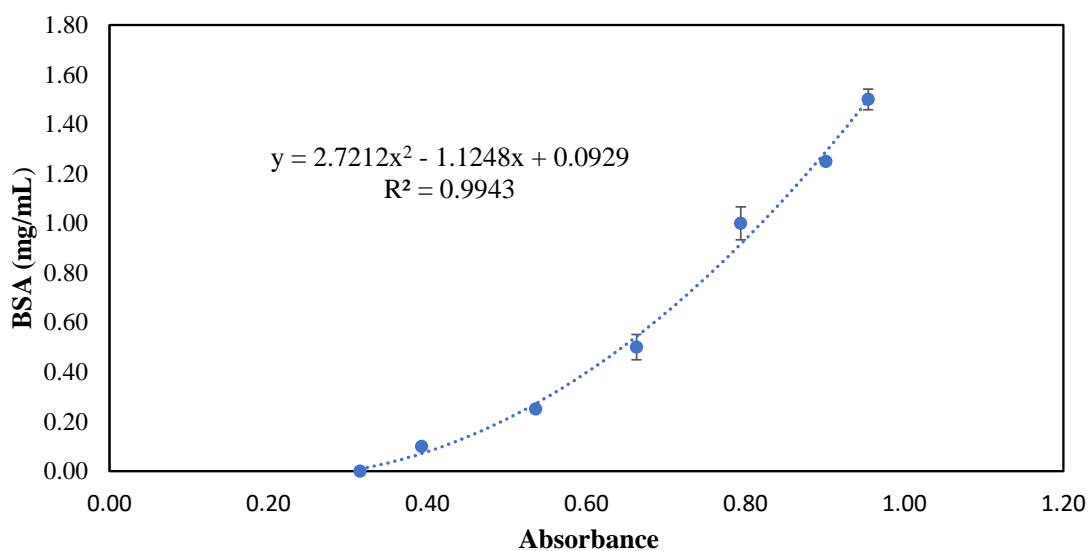
Figure 19. Calibration curve of serum bovine albumin.

3.4. Statistical analysis

Results were expressed as mean values ± 95% confidence interval (95% CI). One-way analysis of variance (ANOVA) was carried out using SPSS software (SPSS Statistical Software, Inc., Chicago, IL, USA). The differences between the pairs of means were evaluated by Tukey's test and the significance level was set at 95%. Each quality analysis was triplicated.

3.5. Experimental design

The following experimental design was carried out for each study:

3.5.1. Study 1: High-pressure Processing (HPP) for decreasing weight loss of fresh albacore (*Thunnus alalunga*) steaks

Albacore samples were subjected to HPP treatments using a wide range of pressures (50–500 MPa) (Figure 20).
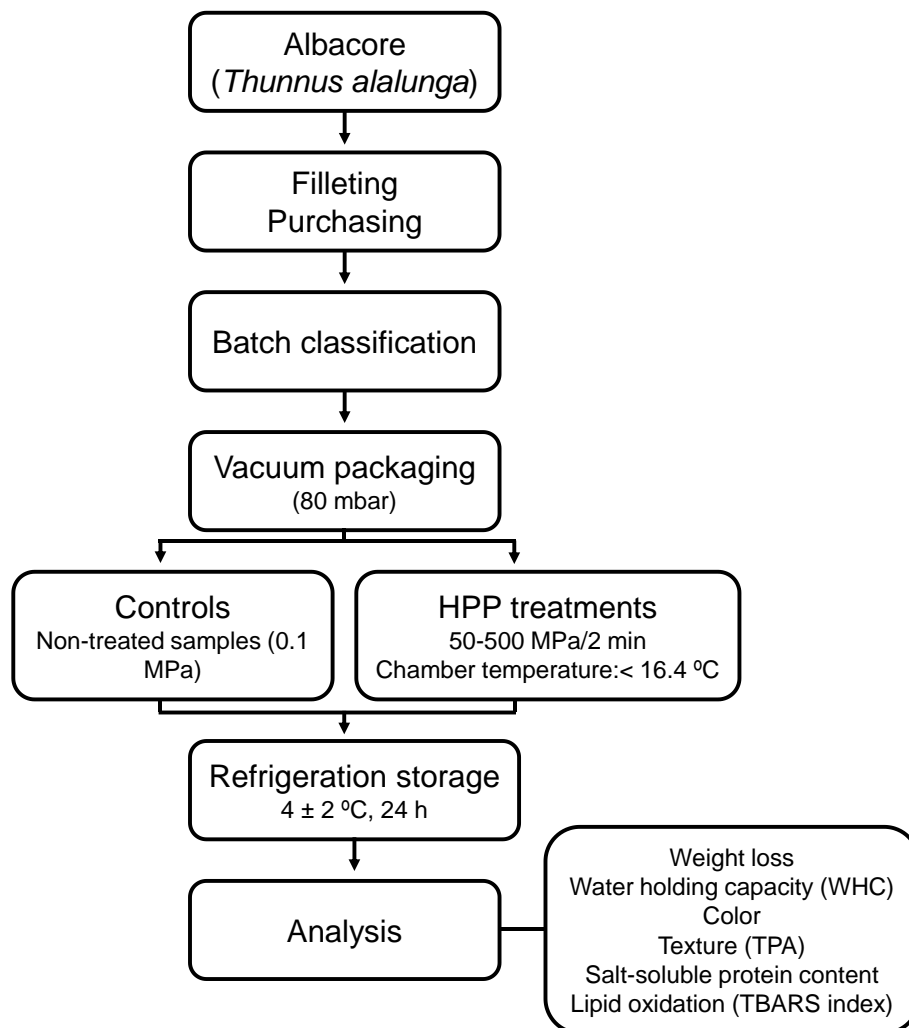
Figure 20. Experimental plan of Study 1.

Pressures of 50, 100, 150, 200, 250, 300, 350, 400, 450 and 500 MPa were selected for 2 min. The times required to reach these pressures ranged from 36 to 190 s. Decompression time was less than 6 s in all cases. 4 albacore steaks were treated for each HPP treatment and 4 non-treated samples were used as control. Once HPP treatments were carried out, all albacore steaks were stored under refrigeration conditions (4 ± 2 ºC during 24 hours)

Then, weight loss, water holding capacity, color, texture (TPA), salt-soluble protein content and lipid oxidation (TBARS index) were analyzed.

3.5.2. Study 2: High-pressure pretreatment in albacore (*Thunnus alalunga*) for reducing freeze-driven weight losses with minimal quality changes

Albacore samples were subjected to HPP pretreatments and then they were subjected to a freeze-thaw process (Figure 21).
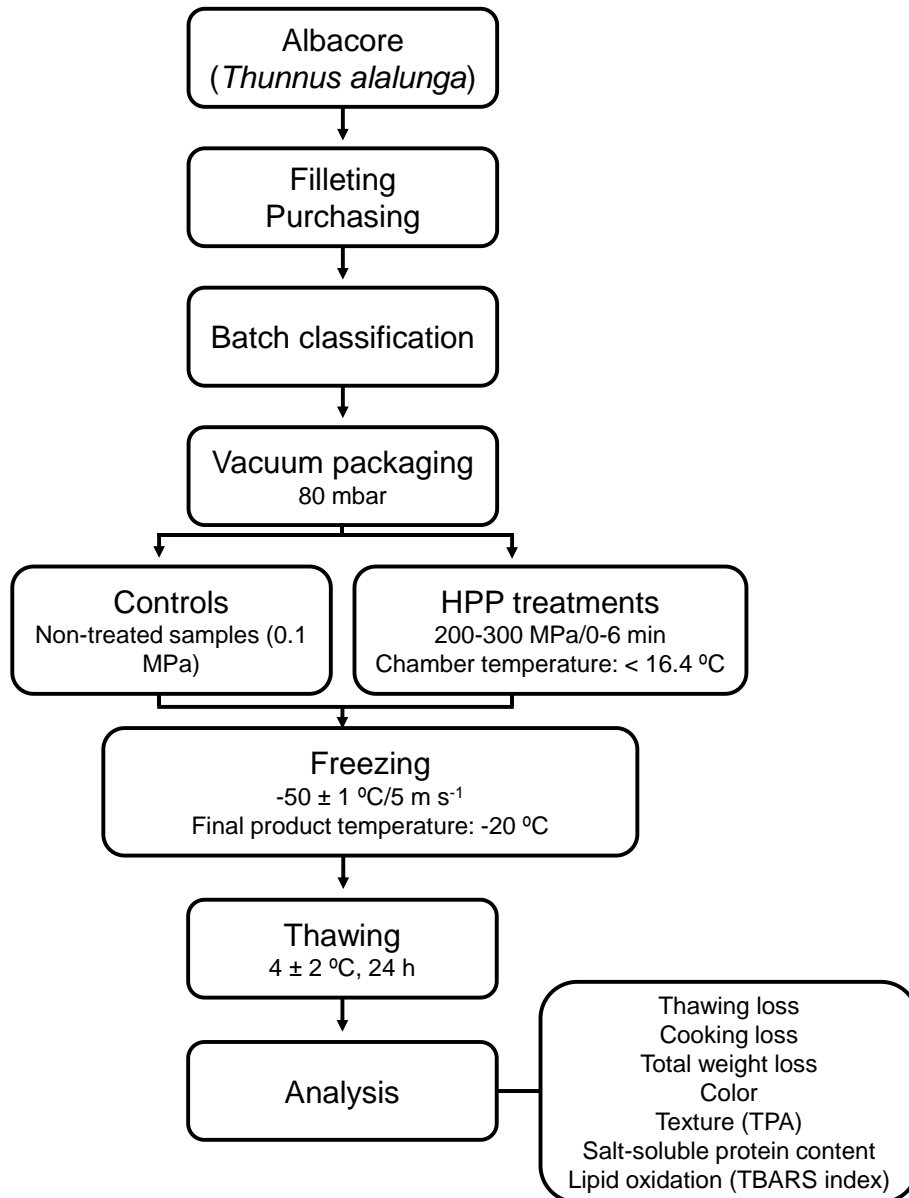
Figure 21. Experimental plan of Study 2.

Pressures of 200, 250 and 300 MPa were applied for 0, 2, 4 and 6 min. The times required to reach these pressures ranged from 102 to 132 s. Decompression time was less than 6 s

in all cases. 3 albacore steaks were treated for each HPP treatment and 3 non-treated samples were used as control. Immediately after HPP pretreatments, all albacore steaks were frozen (-50 ℃, 5 m s^{-1}). When the geometric center of albacore steaks reached -20 ℃, they were immediately thawed (4 ± 2 ℃ during 24 ° hours). Thawing loss, cooking loss, total weight loss, color, texture (TPA), salt-soluble protein content and lipid oxidation (TBARS index) were analyzed once the albacore steaks were thawed.

3.5.3. Study 3: Evolution of quality parameters of high-pressure processing (HPP) pretreated albacore (*Thunnus alalunga*) during long-term frozen storage.

Albacore samples were subjected to HPP pretreatments and then they were subjected to long-term frozen storage (Figure 22).

Pressures of 200 MPa were applied for 0, 2, 4 and 6 min (200/0, 200/2, 200/4 and 200/6, respectively). The times required to reach these pressures ranged from 92 to 98 s. Decompression time was less than 4 s in all cases. For each repetition, 30 albacore steaks were treated for each HPP treatment and 30 non-treated samples were used as control. Immediately after HPP pretreatments, 24 albacore steaks for each HPP pretreatment were frozen (-50 ℃, 5 m s^{-1}). The freezing process was finished when the geometric center of albacore steaks reached -20 ℃. The remaining 6 albacore steaks were stored in a refrigerated chamber (4 ± 2 ℃ during 24 ℃). and were considered as fresh samples. All frozen samples were stored in a freezing chamber (20 ± 2 ℃). For each HPP pretreatment, 6 albacore samples were thawed (4 ± 2 ℃ during 24 hours) after 0, 2, 6 and 12 months, respectively. 3 of these samples were cooked (150 ℃ for 30 min). Then, thawing loss, cooking loss, total weight loss, color, texture (TPA), salt-soluble protein content and lipid oxidation (TBARS index) were determined in both raw and cooked samples.
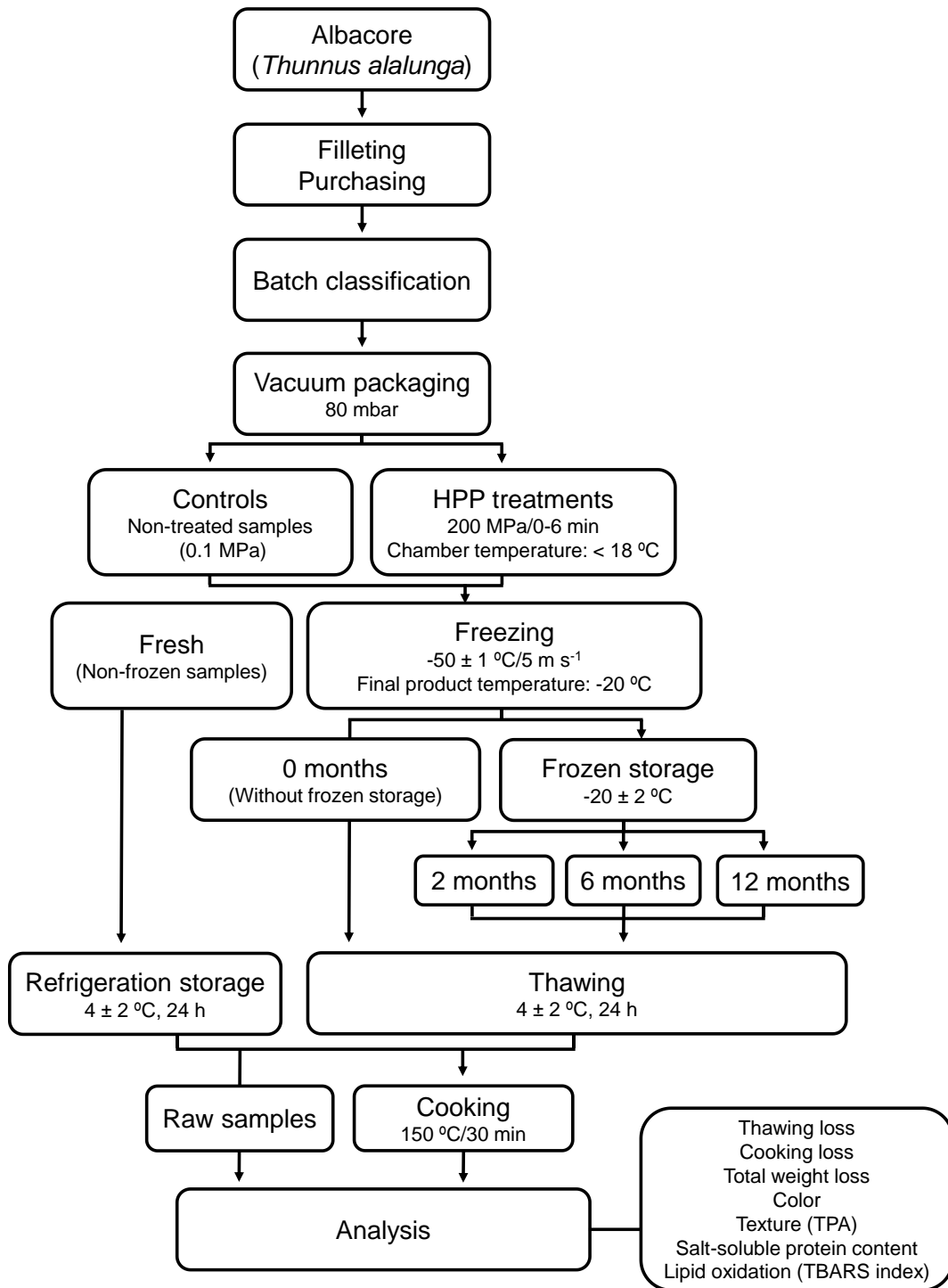
Figure 22. Experimental plan of Study 3.

3.5.4. Study 4: Application of high-pressure processing after freezing (before frozen storage) or before thawing in frozen albacore tuna (*Thunnus alalunga*)

Albacore samples were frozen (-50 ºC, 5 m s^{-1}) and then subjected to HPP pretreatments before frozen storage and before thawing, respectively.

HPP pretreatments were applied at 200 MPa for 6 min and 600 MPa for 0 min. The times required to reach 200 and 600 MPa were 100 and 220 s, respectively. Decompression time was less than 4 s in all cases.

Thawing loss, color, texture (TPA), salt-soluble protein content and lipid oxidation (TBARS index) were determined once the samples were thawed (4 ± 2 ºC during 24 hours).

3.5.4.1. HPP pretreatment before frozen storage

18 albacore steaks were subjected to a freezing process (-50 ºC, 5 m s^{-1}). When the geometric center of albacore steaks reached -20 º C, the freezing process was concluded and samples were immediately subjected to HPP pretreatments (Figure 23).

9 albacore steaks were HPP-pretreated at 200 MPa for 6 min (200/6) and the remaining 9 ones at 600 MPa for 0 min (600/0). Then, all samples were stored in a freezing chamber (-20 ± 2 ºC). 3 albacore samples for each HPP pretreatment were thawed (4 ± 2 ºC during 24 hours) after 0, 15 and 45 days of frozen storage, respectively and then analyzed.
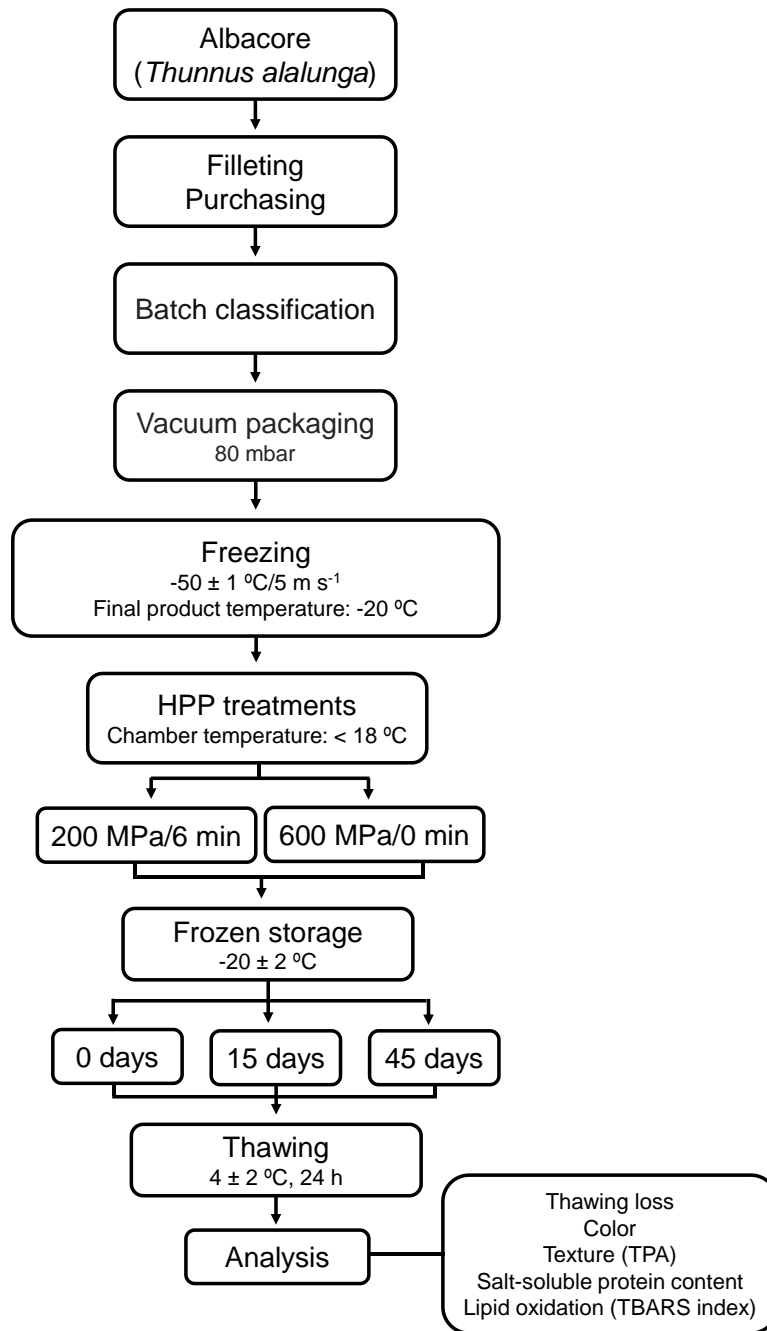
Figure 23. Experimental plan of Study 4: HPP-treated samples before frozen storage (BFS).

3.5.4.2. HPP pretreatment before thawing

18 albacore steaks were subjected to a freezing process (–50 ºC, 5 m s^{-1}). When the geometric center of albacore steaks reached –20 º C, the freezing process was concluded and samples were immediately stored in a freezing chamber (–20 ± 2 ºC). After 0, 15 and 45 days of frozen storage, respectively, 6 albacore steaks were subjected to HPP pretreatments. 3 albacore steaks were HPP-pretreated at 200 MPa for 6 min (200/6) and

the remaining 3 ones at 600 MPa for 0 min (600/0). Then, they were thawed (4 ± 2 ºC during 24 hours) and analyzed (Figure 24).
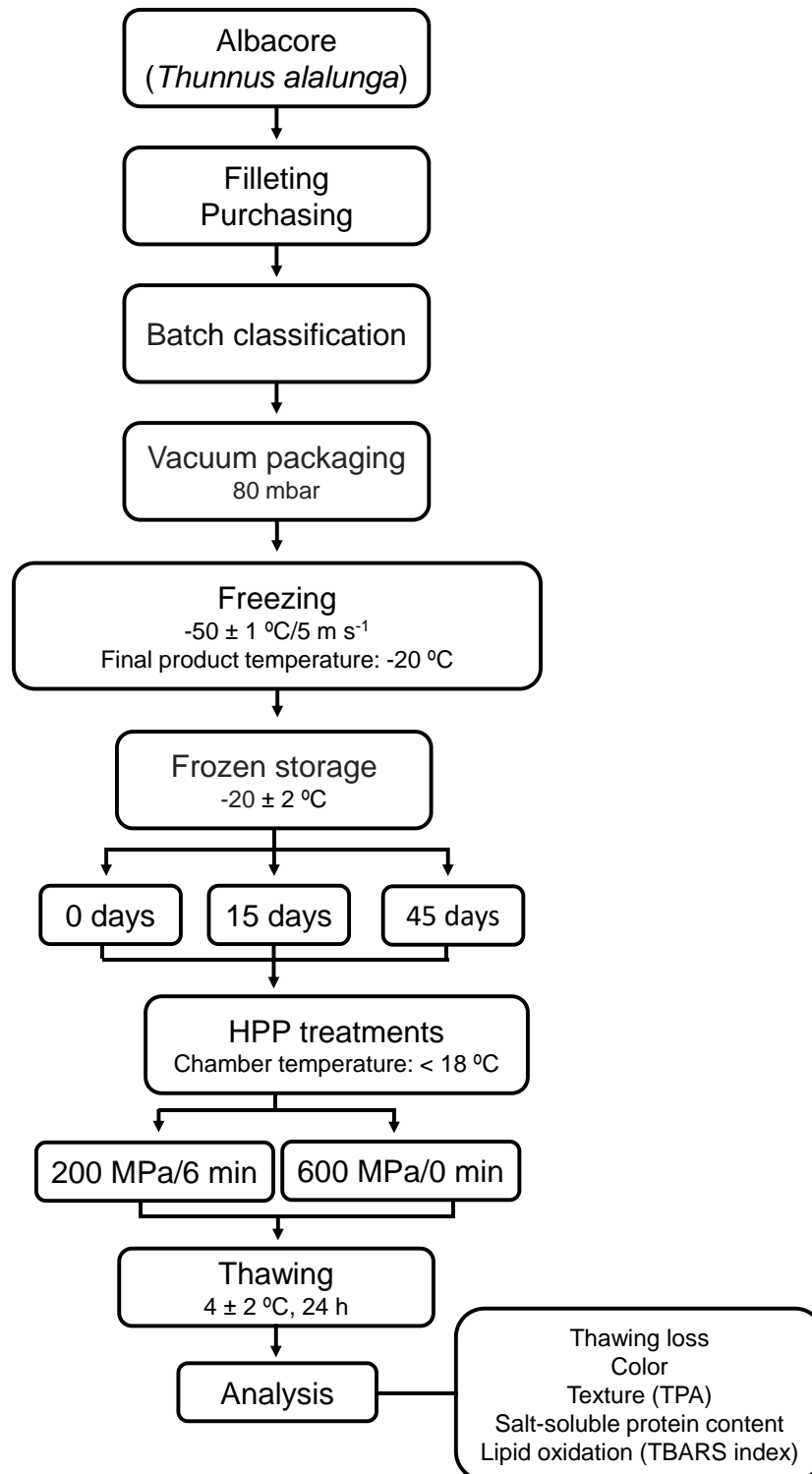
Figure 24. Experimental plan of Study 4: HPP-treated samples before thawing (BT).

3.5.4.3. Control batches

18 albacore steaks were subjected to a freezing process (–50 ℃, 5 m s⁻¹). When the geometric center of albacore steaks reached –20 º C, the freezing process was concluded and the 18 samples were immediately stored in a freezing chamber (–20 ± 2 ℃). during 0, 15 and 45 days (Figure 25). 6 albacore samples were thawed (4 ± 2 ℃ during 24 hours) after 0, 15 and 45 days of frozen storage, respectively and then analyzed.
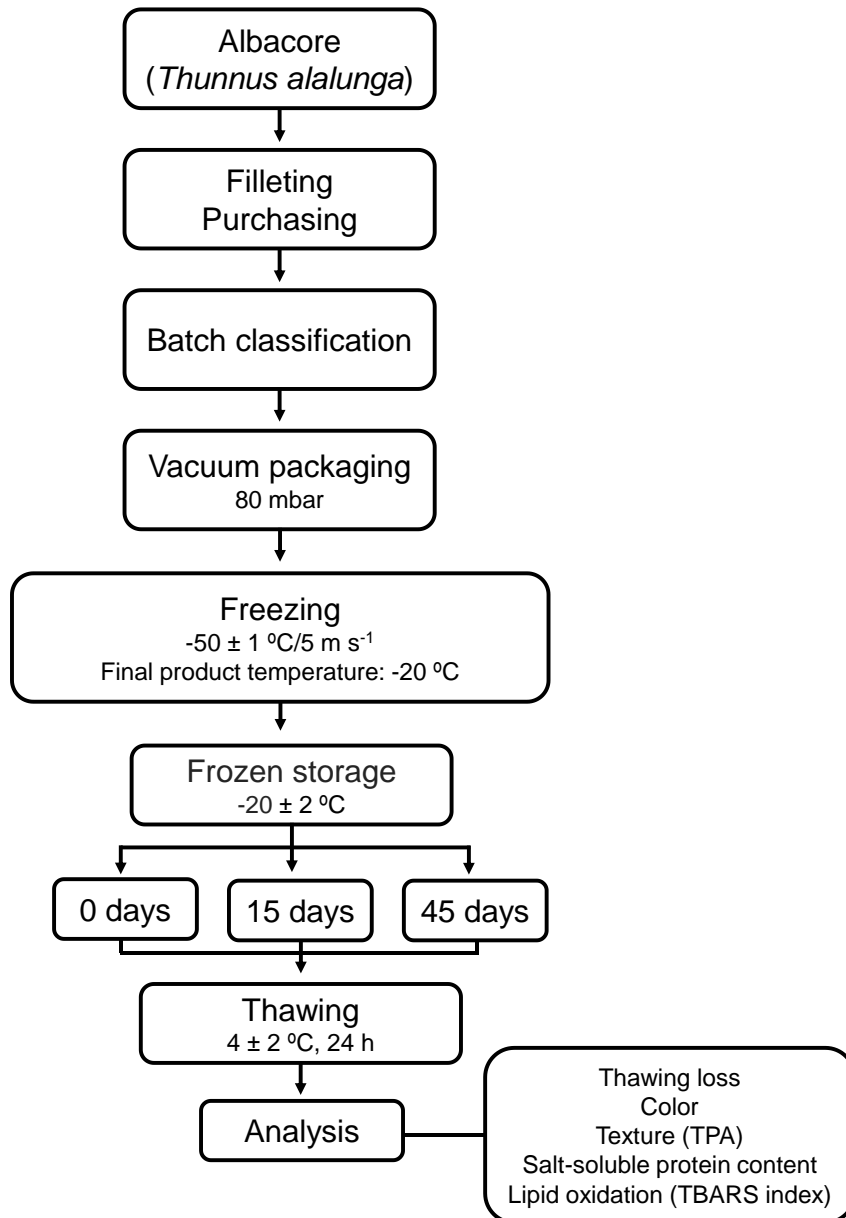
Figure 25. Experimental plan of Study 4: Control samples.

3.5.5. Study 5: Impact of different air blast freezing conditions on the physicochemical quality of albacore (*Thunnus alalunga*) pretreated by high-pressure processing

Albacore samples were subjected to HPP pretreatment and then to different freezing conditions (Figure 26).

24 samples were HPP-pretreated at 200 MPa for 6 min. The time required to reach 200 MPa was less than 100 s. Decompression time was less than 4 s in all cases. 24 non-treated samples were used as control.

Immediately after HPP pretreatments, all albacore steaks were subjected to a freezing process. 4 different freezing conditions were used to carry out the freezing process (–20 $\pm$ 1 ºC and 1 m s^{-1} (20/1), –20 $\pm$ 1 ºC and 5 m s^{-1} (20/5), –50 $\pm$ 1 ºC and 1 m s^{-1} (50/1), and –50 $\pm$ 1 ºC and 5 m s^{-1} (50/5). The initial temperature of the albacore steaks was 5.5 $\pm$ 1 ºC. In all cases, the freezing processes were finished when the geometrical center of albacore steaks reached –20 ºC. 6 HPP-pretreated albacore steaks, as well as 6 control samples were frozen under each freezing condition. Once frozen, all albacore steaks were stored at –20 $\pm$ 2 ºC in a freezing chamber. After 2 and 9 months of frozen storage, both 3 HPP-pretreated samples and 3 controls for each freezing condition were thawed (4 $\pm$ 2 ºC; 24 h) and analyzed.

Then, freezing curves, thawing loss, cooking loss, total weight loss, color, texture (TPA), salt-soluble protein content and lipid oxidation (TBARS index) were determined once the samples were thawed.
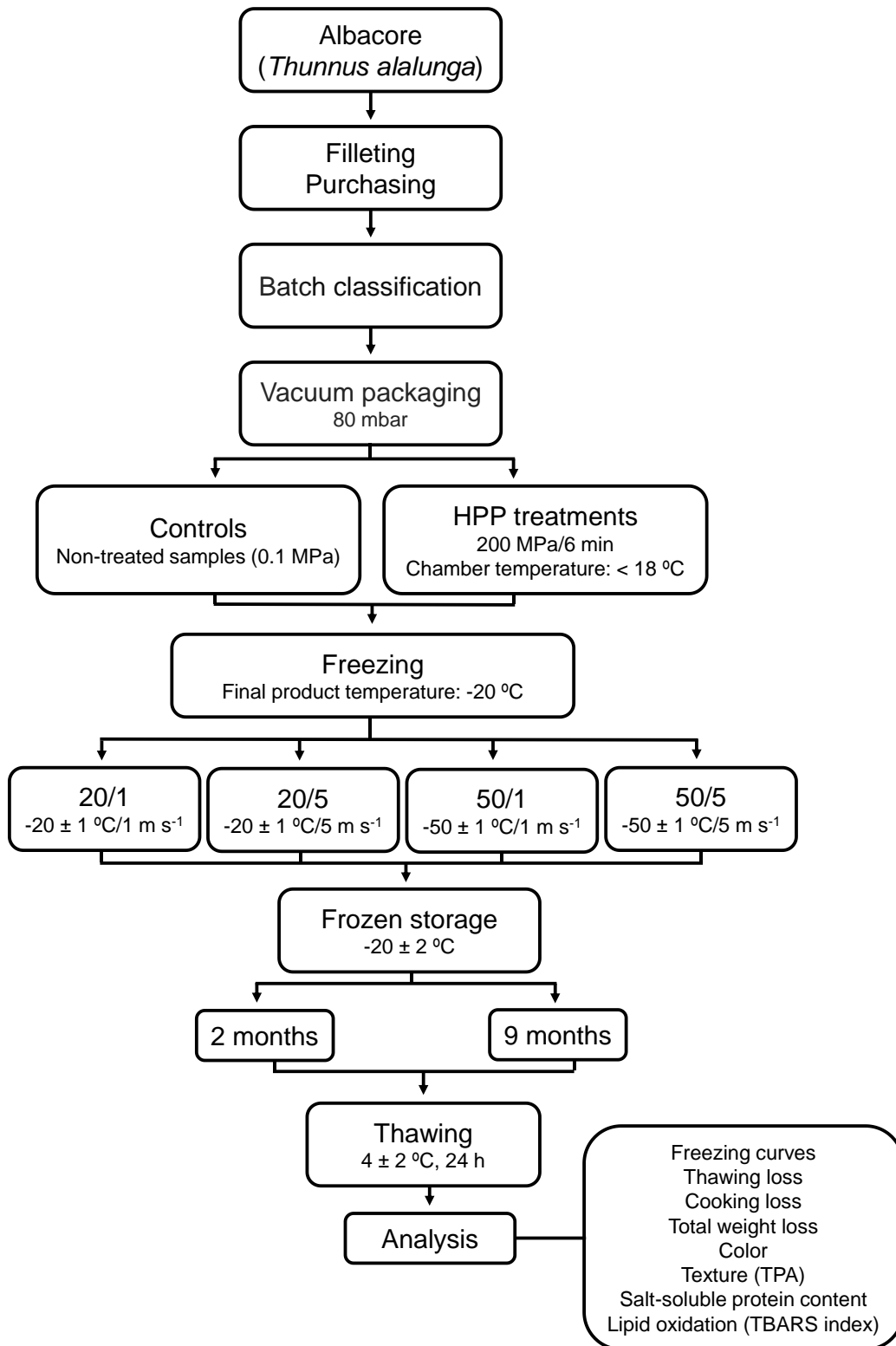
Figure 26. Experimental plan of Study 5.

4.RESULTS AND DISCUSSION

4.1. Study 1: High-pressure Processing (HPP) for decreasing weight loss of fresh albacore (*Thunnus alalunga*) steaks

In the present work, a comprehensive study on the application of high-pressure processing (HPP) treatments on fresh albacore (*Thunnus alalunga*) was carried out.

Weight loss is a very important issue during processing due to economic losses as well as loss of water-soluble nutrients and its negative impact on appearance and texture (Christensen et al., 2017). Thus, research has been focused on the development of innovative technologies for decreasing while retaining fresh-like quality. In this sense, high-pressure processing (HPP) is an alternative non-thermal technology that has shown potential to improve the water retention of muscle tissues (Chéret et al., 2005) an subsequently, to decrease weight loss (Souza et al., 2011; Xuan et al., 2018) in fish products.

However, HPP treatment induces undesirable changes in color and texture (Suemitsu & Cristianini, 2019; Yagiz et al., 2009) that are more pronounced as pressure increases (Chouhan et al., 2015; Christensen et al., 2017; Ramirez-Suarez & Morrissey, 2006; Yagiz et al., 2009).

Hence, this work was focused on finding the optimal HPP treatment for decreasing weight loss of fresh albacore (*Thunnus alalunga*) while retaining as much as possible the inherent color and texture characteristics of fresh fish.

4.1.1. Weight loss

In the control albacore, there was a weight loss of $1.38 \pm 0.19\%$. Similar weight loss values were obtained in bluefin tuna after 2 days of refrigerated storage (W. Jiang et al., 2019). Weight loss of albacore steaks increased at pressures from 50 to 150 MPa compared to the control (Figure 27a), reaching a value of $2.24 \pm 0.36\%$ at 150 MPa. However, a progressive decreasing trend in weight loss was observed above 200 MPa. Treated samples at 200 MPa showed a 41.6% decrease in weight loss in comparison with treated samples at 150 MPa. At 250 MPa, weight loss decreased by 50.1% compared with the control samples. Treated Albacore at 500 MPa showed the highest decrease in weight loss, reaching a value of $0.56 \pm 0.11\%$ (59.4% decrease in comparison with the control samples). These results were in accordance with the results reported by Souza et al. (2011) in treated samples of *longissimus dorsi* and *semimembranosus* pork muscles at 215 MPa for 15 s at 33.3 °C, in which drip loss was decreased from 2.16 and 2.13% to 0.30 and 0.33%, respectively, as a result of the HPP treatment. However, no effect on weight loss

of HPP treatment was observed in sea bass (*Dicentrarchus labrax* L.) and cod (*Gadus morhua*) (Chéret et al., 2005; Christensen et al., 2017), and a higher weight loss was found in Atlantic mackerel (*Scomber scombrus*) after HPP treatment (200 and 500 MPa for 2 min at 8–9 °C) (Christensen et al., 2017). These different behaviors would be related to the significant effect of fish species on the impact of HPP (Barba et al., 2015b), highlighting the necessity of individual studies for optimizing HPP treatments. In this regard, the muscle structure of albacore is more similar to white meat than to white fish species, being more stable to protein denaturation and degradation (Venugopal, 2005a).

The progressive increase of weight loss at low pressure levels (50–150 MPa) found in the present work could be related to the changes in tissue architecture, the increase of cell permeabilization, and the consequent output of intracellular liquid (Rastogi et al., 2007). At pressure levels between 150 and 250 MPa, a progressive reduction of weight loss was obtained. This can be attributable to the HPP-mediated changes in protein, such as denaturation, aggregation, and gelatinization (Alves de Oliveira et al., 2017; Rastogi et al., 2007). Above 250 MPa, weight loss reduction was maintained constant. This could be due to protein changes induced by HPP treatment around 250 MPa become irreversible, and hence, weight loss does not decrease much more as the pressure increases (Chéret et al., 2005). Similar pressures to those which decreased weight loss in this work (200 MPa) led to extend shelf life of yellowfin tuna (*Thunnus albacares*) in chilled storage up to 10 days with respect to the untreated samples (Kamalakanth et al., 2011). Longer shelf life was also found in HPP-treated minced albacore (275–310 MPa for 2–6 min) in comparison with the control albacore (Ramirez-Suarez & Morrissey, 2006).
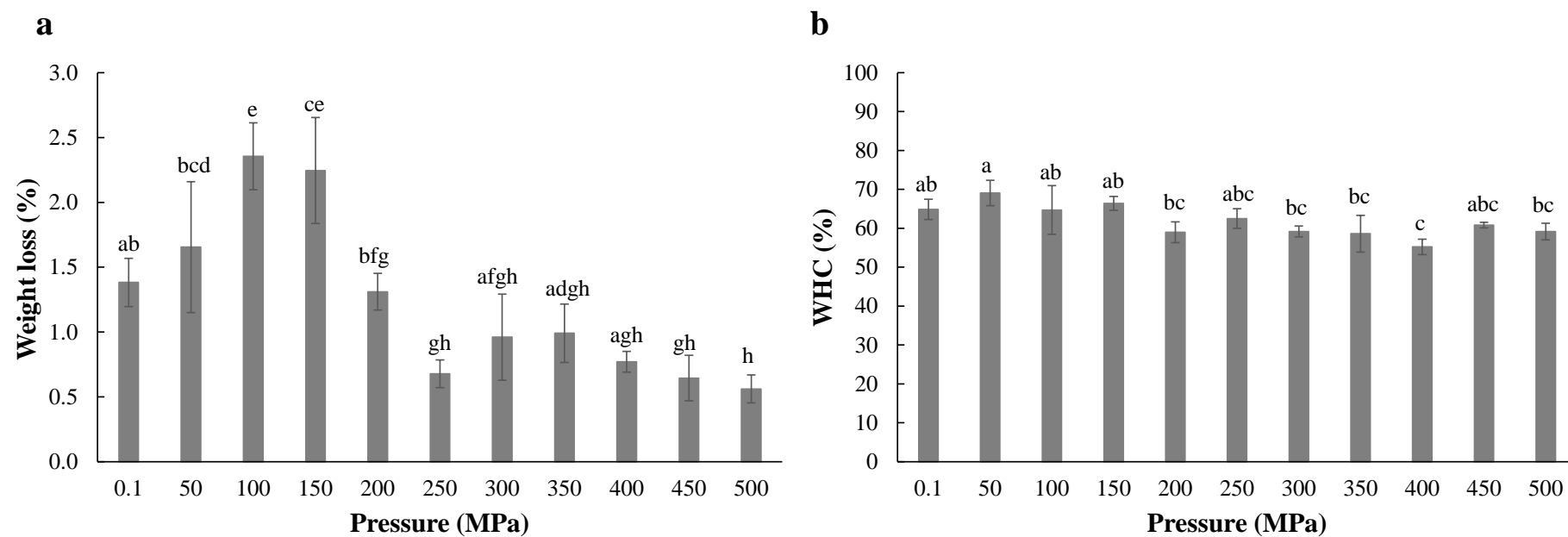
Figure 27. Weight loss (%) (a) and water holding capacity (%) (b) of fresh albacore steaks after HPP treatment (50–500 MPa). The results of control samples (0.1 MPa) were also shown. Error bars indicate 95 CI. Different letters indicate significant differences ($p \leq 0.05$) as a result of the pressure applied.

4.1.2. Water holding capacity

HPP treatment had no effect on the WHC of albacore steaks since only HPP-treated albacore at 400 MPa showed significantly lower values ($p \leq 0.05$) than the control albacore (Figure 27b). These results are in agreement with those reported by Christensen et al. (2017). These authors did not observe significant changes in WHC compared with control samples after 0 days of refrigerated storage in HPP-treated cod, Atlantic mackerel and Atlantic salmon (*Salmo salar*) at 200 and 500 MPa for 2 min at 8–9 °C. Gómez-Estaca et al. (2007) reported that treated dolphinfish (*Coryphaena hippurus*) at 400 Ma for 15 min at 20 °C showed significantly lower WHC ($p \leq 0.05$) than control samples, while there were no significant changes at lower pressures. Other authors reported that WHC significantly decreased as a result of HPP treatment (Gómez-Estaca et al., 2009; Jiranuntakul et al., 2018). The WHC of control samples was slightly lower than the values determined by Gómez-Estaca et al. (2009) in untreated albacore carpaccio. However, WHC of treated albacore at 200 and 250 MPa for 2 min was similar to that determined in treated albacore carpaccio at 200 MPa and 250 MPa for 15 min at 7 °C (Gómez-Estaca et al., 2009), while WHC of treated samples at 150 MPa for 2 min was similar to that determined by Jiranuntakul et al. (2018) in treated skipjack tuna (*Katsuwonus pelamis*) at 150 MPa for 3 min at room temperature. Decrease in WHC could be attributed to muscle protein denaturation, which would reduce water-protein interactions (Gómez-Estaca et al., 2009).

4.1.3. Color

Figure 28 shows L^* (a), a^* value (b), b^* value (c), and total color difference (ΔE; d) of white and red muscle of albacore steaks after HPP treatment (50–500 MPa for 2 min). HPP treatment increased the L^* value of the white muscle of albacore with increasing pressure, leading to significant changes ($p \leq 0.05$) above 200 MPa when compared to control samples (Figure 28a). Other authors also found an increasing trend in L^* value due to the HPP treatment in hilsa (*Tenualosa ilisha*), cod, Atlantic salmon, albacore, hake (*Merluccius merluccius*), and tilapia (*Oreochromis niloticus*) (Chouhan et al., 2015; Christensen et al., 2017; Ramirez-Suarez & Morrissey, 2006; Suemitsu & Cristianini, 2019; Yagiz et al., 2009). Actin and myosin denaturation due to HPP treatment could lead to an increased light reflectance of muscle, resulting in an increment of L^* value (Chouhan et al., 2015).
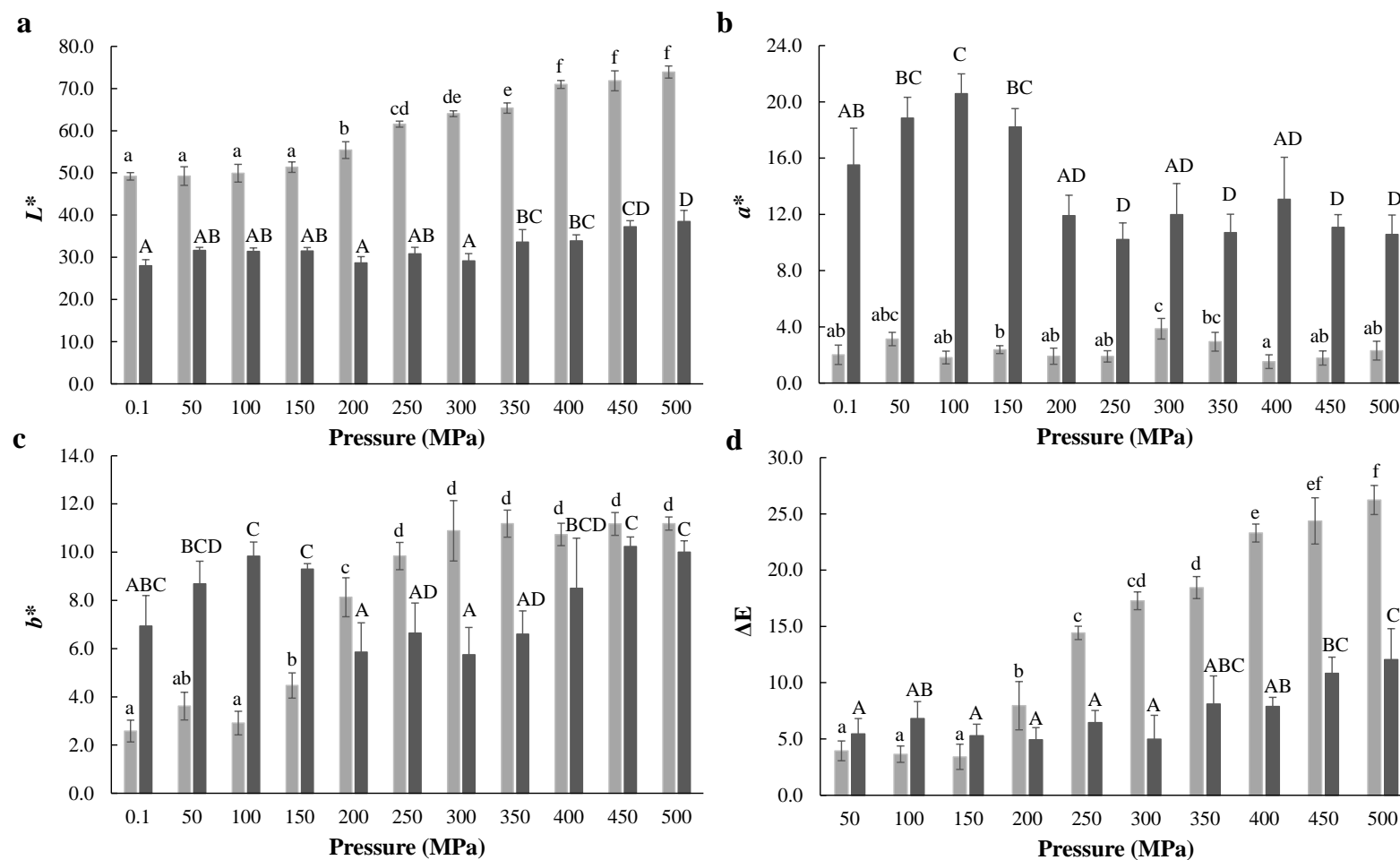
Figure 28. *L** (a), *a** value (b), *b** value (c), and total color difference (d) of white (light gray bars) and red muscle (dark gray bars) of fresh albacore steaks after HPP treatment (50–500 MPa). The results of control samples (0.1 MPa) are also shown. Errors bars indicate 95 CI. Different letters indicate significant differences ($p \leq 0.05$) as a result of the pressure applied for white (lowercase letters) and red (capital letters) muscle.

The red muscle of albacore steaks, which is rich in myoglobin, showed an increase in L^* value due to HPP treatment. However, significant differences with respect to control samples were only observed in HPP-treated samples above 350 MPa. Similar results were observed in beef, a food product with high myoglobin content (Jung et al., 2003; H. J. Kim et al., 2014; Marcos et al., 2010). The increase in L^* value of red muscle as a result of HPP treatment could be attributed to globin denaturation and heme group displacement or release (Marcos et al., 2010).

No effect of HPP treatment on the a^* value of the white muscle of albacore steaks was observed (Figure 28b). There were some significant differences depending on the pressure applied, although a clear tendency cannot be concluded. Similar results were obtained by other authors (Aubourg, Rodríguez, et al., 2013; Gómez-Estaca et al., 2009; Hughes et al., 2016).

Gómez-Estaca et al. (2009) reported that a^* value is highly dependent on the species and the HPP treatment conditions. The white muscle of albacore does not have a high content of myoglobin, which is responsible for the redness of meat and, consequently, determines the a^* value. This could justify the lack of a clear effect of pressure in the a^* value of white muscle.

Related to the a^* value of red muscle, there was an initial increase in the HPP-treated samples, although significant differences ($p \leq 0.05$) were only detected in treated samples at 100 MPa in comparison with control samples (Figure 28b). Above 150 MPa, the a^* value started to decrease, reaching similar values than the control samples at 150 and 200 MPa. The a^* value of HPP-treated samples at 250, 350, 450, and 500 MPa was significantly lower than those of the control samples. Similar changes were reported by Jung et al. (2003), who observed an initial increase in a^* value in HPP-treated beef muscle up to 350 MPa for 4 min at 10 °C and then a decrease between 350 and 600 MPa for 4 min at 10 °C. A decrease in a^* value due to HPP treatment was also reported by other authors in beef (H. J. Kim et al., 2014; Marcos et al., 2010). It has been suggested that the enzymatic system involved in metmyoglobin reduction could be activated at lower pressures leading to a decrease of metmyoglobin content and, consequently, to an increase in a^* value (Jung et al., 2003). However, at higher pressures, ferrous myoglobin oxidation to ferric metmyoglobin due to HPP treatment could involve a decrease in a^* value (Carlez et al., 1995; Marcos et al., 2010).

The $b*$ value of white muscle of albacore steaks increased with increasing pressure, although significant changes ($p \leq 0.05$) were detected above 150 MPa in comparison with control samples, according to the pressure from which weight loss started to decrease in comparison with control samples (Figure 28c). There were no significant changes ($p > 0.05$) in $b*$ value between 250 and 500 MPa. In tilapia, $b*$ value significantly increased at HPP treatments above 300 MPa for 1 min at 5 °C (Suemitsu & Cristianini, 2019). An increase in $b*$ value was also reported in turbot (*Scophthalmus maximus*) and hilsa as a result of HPP treatment of up to 200 MPa for 30 min at 4 °C and 350 MPa for 10 min at room temperature, respectively (Chevalier et al., 2001; Chouhan et al., 2015). However, $b*$ value decreased in Coho salmon (*Oncorhynchus kisutch*) and after HPP treatment, independently of the applied pressure (135 to 200 MPa for 30 s at 15 °C) (Aubourg, Rodríguez, et al., 2013). Changes in $b*$ value could be related to lipid oxidation development (Aubourg, Rodríguez, et al., 2013).

The $b*$ value of red muscle was significantly higher ($p \leq 0.05$) in HPP-treated samples at 100, 150, 450, and 500 MPa when compared to control samples. A significant increase in $b*$ value was also reported in treated beef above 400 MPa for 20 min at 10, 20, and 30 °C (Marcos et al., 2010).

The total color difference (ΔE) of the white muscle of albacore steaks gradually increased with pressure (Figure 28d). A rise of 10 units of ΔE, which is considered to modify significantly the appearance of meat (Jung et al., 2003), was reached above 250 MPa. Similar results were obtained in turbot and hilsa (Chevalier et al., 2001; Chouhan et al., 2015). Since noticeable changes in color parameters took place around 150–250 MPa and weight loss started to decrease at 200 MPa, the most adequate treatment could be ranged between 200 and 250 MPa.

In red muscle, ΔE also increased with the pressure increment, although this was not as remarkable as in the white muscle. Albacore red muscle pressurized at 450 MPa and 500 MPa showed the highest increase in ΔE, reaching values above 10 units. Similar results were obtained in treated beef above 400 MPa for 20 min (Marcos et al., 2010).

4.1.4. Appearance

Albacore steaks still retained their characteristic appearance after HPP treatment up to 200 MPa (Figure 29). Above 250 MPa, the white muscle of albacore steaks became

lighter and more opaque, while negligible changes were detected in the red muscle. Above 350 MPa, albacore steaks had a cooked-like appearance. Similar results were observed in barramundi (*Lates calcarifer*), cod, tilapia, and sea bass (Angsupanich & Ledward, 1998; Suemitsu & Cristianini, 2019; Teixeira et al., 2014; Truong et al., 2016). Chouhan et al. (2015) observed that treated hilsa at 250 MPa for 10 min at room temperature was similar to the control samples, while HPP-treated samples at 350 MPa for 10 min at room temperature became lighter and more opaque, being similar to cooked samples. Suemitsu & Cristianini (2019) carried out a sensorial analysis to evaluate the appearance of HPP-treated tilapia steaks from 100 to 400 MPa for 3 min at 5 °C. These authors observed that liking scores of treated samples at 200 MPa were similar to those of the controls.

Changes in appearance were in accordance with the results observed in color measurements, where L^* and b^* values of white muscle were significantly higher than those of controls in HPP-treated samples above 200 MPa and 150 MPa for 2 min, whereas a^* value of red muscle significantly decreased above 250 MPa for 2 min. Furthermore, remarkable changes in appearance of albacore steaks started to occur above 250 MPa, concurrently with the significant decrease in weight loss compared to untreated samples.
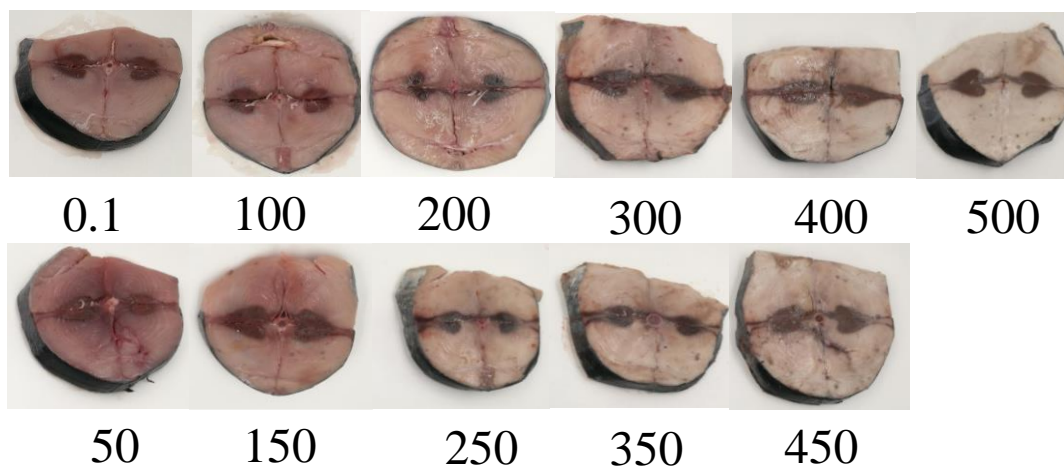
Figure 29. Albacore steaks after HPP treatment from 50 MPa to 500 MPa for 2 min. The appearance of control samples (0.1 MPa) was also shown.

4.1.5. Texture (TPA)

Figure 30 shows hardness (a), adhesiveness (b), springiness (c), and chewiness (d) of albacore steaks after HPP treatment (50–500 MPa for 2 min). After HPP treatment, hardness reached 4.4 ± 0.6 and 8.2 ± 1.3 N in treated samples at 250 and 300 MPa,

respectively, while control samples resulted in a hardness of 3.4 ± 0.5 N (Figure 30a). Similar values were obtained in treated minced albacore under similar conditions of pressure and time (around 4 N at 275 MPa and around 7 N at 310 min for 2 min) immediately after treatment (Ramirez-Suarez & Morrissey, 2006). No effect of HPP treatment was observed at lower pressures (≤ 200 MPa). However, at higher pressures (≥ 250 MPa), hardness increased with increasing pressure, showing significantly higher values ($p \leq 0.05$) than control samples. Moreover, significant differences ($p \leq 0.05$) were observed among treatments. Treated samples at 500 MPa showed a remarkable increase ($p \leq 0.05$) in hardness compared to treated samples at 450 MPa. Jiranuntakul et al. (2018) observed that HPP treatment up to 600 MPa for 1, 3, and 5 min at room temperature resulted in a significant increase in hardness of skipjack tuna compared to raw samples. (Chouhan et al. (2015) also reported in hilsa steaks an increasing trend with increasing pressure immediately after HPP treatment up to 350 MPa for 10 min at room temperature. By contrast, no significant changes in hardness due to HPP treatment were reported in tilapia and abalone (*Haliotis rufescens*) (Hughes et al., 2016; Suemitsu & Cristianini, 2019). The increase in hardness could be justified by the protein denaturation which would take place around 200–250 MPa and which would also be related to the decrease in weight loss and color changes (Alves de Oliveira et al., 2017).

Adhesiveness of albacore steaks started to increase at 200 MPa and then decreased at 300 MPa (Figure 30b). Processed samples between 250 and 350 MPa showed a significantly higher adhesiveness ($p \leq 0.05$) than control samples. These results are in agreement with Yagiz et al. (2009) who observed in HPP-treated Atlantic salmon at 300 MPa for 15 min at room temperature an increase in adhesiveness with respect to control samples. Torres et al. (2014) observed at time 0 of frozen storage that adhesiveness of horse mackerel *(Trachurus trachurus)* increased due to the HPP pretreatment applied before freezing (up to 450 MPa for 5 min at room temperature). The increase in adhesiveness between 250 and 350 MPa could be caused by the unfolding of actin and sarcoplasmic proteins and the formation of hydrogen-bonded networks (Angsupanich & Ledward, 1998).

Angsupanich & Ledward (1998) observed by differential scanning calorimetry (DSC) that the peak corresponding to myosin had disappeared after HPP treatment at 200 MPa for 20 min at room temperature, coinciding with a decrease in adhesiveness with respect to the control, whereas the peaks corresponding to actin and sarcoplasmic proteins had disappeared after HPP treatment at 300 MPa for 20 min at room temperature, coinciding

with an increase in adhesiveness. Christensen et al. (2017) also observed that the peak corresponding to myosin disappeared after HPP treatment at 200 MPa for 2 min at 8–9 °C in cod and Atlantic mackerel, while further pressure was required for actin denaturation.

All treated albacore samples, excluding those treated at 50 and 150 MPa, showed significantly higher springiness ($p \leq 0.05$) than the controls (Figure 30c). These results are in accordance with Chouhan et al. (2015), who observed that the springiness of treated hilsa steaks at 300 MPa for 10 min at room temperature was significantly higher than that of the control samples at day 0 of refrigerated storage, while no differences were found at 150 MPa for 10 min at room temperature. Springiness of skipjack tuna decreased at low pressures (150–300 MPa, up to 3 min), while it increased at higher pressures (400–600 MPa, up to 3 min) compared to control samples (Jiranuntakul et al., 2018).

Chewiness gradually increased with pressure (Figure 30d). However, there were no significant differences ($p > 0.05$) between HPP-treated samples below 200 MPa and the control samples. A similar trend was reported in several fish species (Angsupanich & Ledward, 1998; Chouhan et al., 2015; Yagiz et al., 2009).

The hardness, springiness, and chewiness of HPP-treated albacore changed in the same manner with the pressure. Thus, the unfolding of actin and sarcoplasmic proteins and the formation of hydrogen-bonded networks due to HPP treatment could be involved in these changes (Angsupanich & Ledward, 1998; Chouhan et al., 2015).

In conclusion, noticeable changes in texture parameters of albacore took place above 200–250 MPa, while weight loss started to decrease above 200 MPa. This could be explained by the protein denaturation due to HPP treatment, which affects both weight loss and texture of albacore steaks.
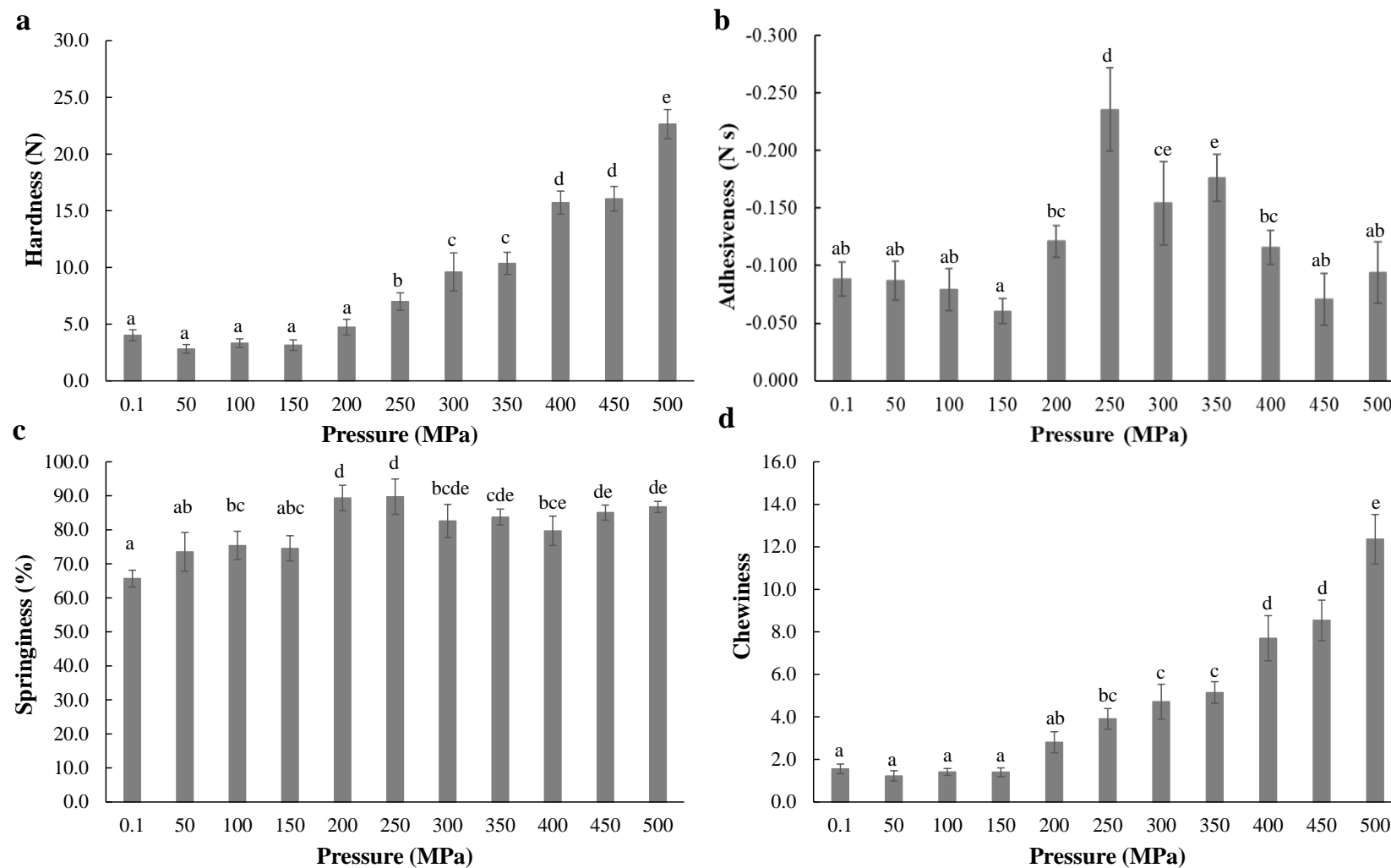
Figure 30. Hardness (a), adhesiveness (b), springiness (c), and chewiness (d) of fresh albacore steaks after HPP treatment (50–500 MPa for 2 min). The texture parameters of control samples (0.1 MPa) are also shown. Error bars indicate 95 CI. Different letters indicate significant differences ($p \leq 0.05$) as a result of the pressure applied.

4.1.6. Salt-soluble protein content

Figure 31 shows the salt-soluble protein content of albacore steaks after HPP treatment (50–500 MPa for 2 min). Salt-soluble protein index indicates denaturation and aggregation of muscle proteins extracted in a high ionic strength solution (0.6 M), which is predominated by myofibrillar proteins but may also contain sarcoplasmic proteins (Venugopal, 2005a). Salt-soluble protein decreased as the pressure level increased. Treated samples above 150 MPa showed a significantly lower ($p \leq 0.05$) level than the control samples. There were no significant differences ($p > 0.05$) in treated samples between 150 MPa and 350 MPa. Treated albacore at 500 MPa showed the lowest salt-soluble protein content. Ko et al. (2006) also observed in HPP-treated tilapia that salt-soluble protein decreased with increasing pressure above 200 MPa for 1 h at 25 °C, whereas there was no effect of HPP treatment on water-soluble protein (mainly sarcoplasmic proteins). Decrease in salt-soluble protein above 200 MPa could be related to actomyosin coagulation due to pressure treatment (Ko et al., 2006). A progressive decrease in sarcoplasmic protein with increasing pressure was observed in treated Coho salmon from 135 to 200 MPa for 30 s at 15 °C (Ortea et al., 2010). Méndez et al. (2017) did not observe significant changes in sarcoplasmic and myofibrillar protein content in sardine (*Sardina pilchardus*) at time 0 of frozen storage as a result of HPP pretreatment (125–200 MPa for 0 min at 20 °C) before freezing, although they reported an inverse correlation among myofibrillar protein content and the pressure applied. Thus, higher pressures could result in significant changes in salt-soluble protein content.
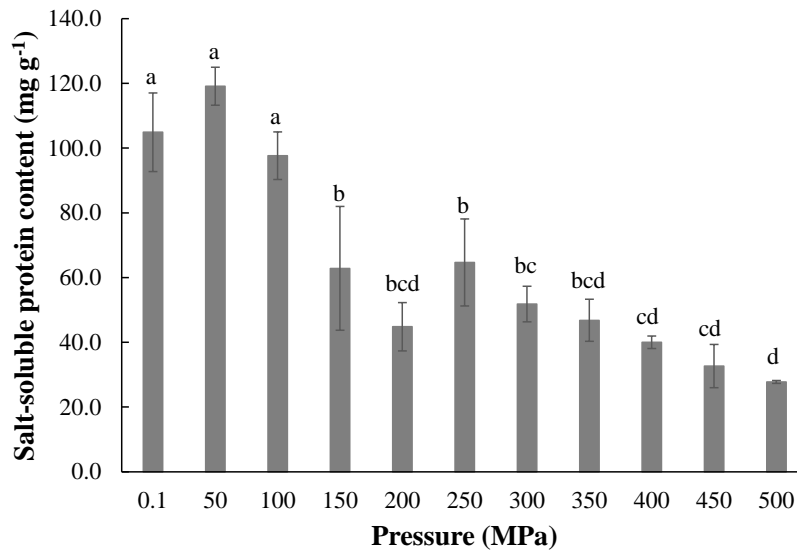
Figure 31. Salt-soluble protein of fresh albacore steaks after HPP treatment (50–500 MPa for 2 min). Salt-soluble protein content of control samples (0.1 MPa) is also shown. Error bars indicate 95 CI. Different letters indicate significant differences ($p \leq 0.05$) as a result of the pressure applied.

4.1.7. TBARS value

TBARS values after HPP treatment (50–500 MPa for 2 min) are shown in Figure 32. Control samples presented a TBARS value of 1.62 ± 0.27 mg MDA kg^{-1}. Similar values were detected at day 0 of refrigerated storage in non-pressurized minced albacore samples (Ramirez-Suarez & Morrissey, 2006). This content was markedly higher than values detected in other fish species, such as hilsa or sardine (Chouhan et al., 2015; Méndez et al., 2017), although it was below the acceptability limit referenced by Silbande et al. (2016) in yellowfin tuna (5–8 mg MDA kg^{-1}). Moreover, albacore presents a high amount of red muscle, which has a high pro-oxidant substance content, such as iron, hemoglobin, or myoglobin (Alves de Oliveira et al., 2017). Thus, albacore could be highly susceptible to lipid oxidation.
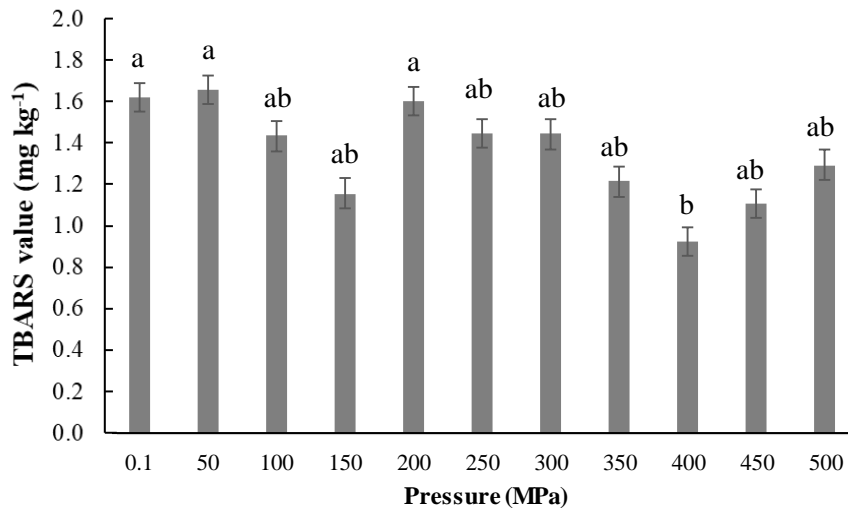
Figure 32. TBARS value of fresh albacore steaks after HPP treatment (50–500 MPa for 2 min). TBARS value of control samples (0.1 MPa) is also shown. Error bars indicate 95 CI. Different letters indicate significant differences ($p \leq 0.05$) as a result of the pressure applied.

In accordance with Chouhan et al. (2015) and Yagiz et al. (2009), a clear effect of HPP treatment on lipid oxidation could not be concluded. Only albacore treated samples treated at 400 MPa for 2 min showed lower ($p \leq 0.05$) TBARS values than the controls. Other authors observed an increase in TBARS value due to HPP treatment (Angsupanich & Ledward, 1998; Chevalier et al., 2001; Gómez-Estaca et al., 2009) that could be attributed to the release of heme proteins and the disruption of lipid membranes (Barba et al., 2015b). By contrast, Ramirez-Suarez & Morrissey (2006) did not find differences in TBARS value between HPP-treated minced albacore (up to 310 MPa for 2–6 min at 10 °C) and the control samples at day 0 of refrigerated storage. Thus, it could be concluded that TBARS value is highly dependent on fish species, HPP treatment conditions (pressure, temperature, and time), and sample handling (catching and transport conditions and presentation mode) (Barba et al., 2015b; Otero et al., 2019; Ramirez-Suarez & Morrissey, 2006).

According to bibliography, HPP has no relevant effects on total lipid content and fatty acid profile in oily fish species like albacore. For example, no differences in total lipid content between the control and HPP-treated samples at similar conditions to those employed in the present work (135–200 MPa for 30 s) were found in Coho salmon (Ortea et al., 2010). Regarding the fatty acid profile, other authors reported no differences between HPP-treated Atlantic salmon (150 and 300 MPa for 15 min) and the control in total saturated, monoenes, n−3PUFA, and n−6PUFA fatty acid compositions (Yagiz et

al., 2009). Similarly, no differences were found in the fatty acid profile of beef as a consequence of intense HPP treatments (200–400 MPa for 20 min) (McArdle et al., 2010).

This work shows that HPP treatment at 500 MPa for 2 min would lead to the maximum reduction of weight loss (59.4% decrease in comparison with control samples) and, consequently, decrease economic losses during the processing of albacore. However, this HPP treatment caused marked differences in color (significantly higher L^* and b^* values in both white and red muscle and lower a^* value in red muscle than the control samples, respectively, and ΔE higher than 10 units in both white and red muscle) and texture (significantly higher hardness, springiness, and chewiness than the control samples) which could affect consumer acceptance. Weight loss of albacore steaks started to decrease around 200 MPa (5.2% decrease in comparison with control samples). At 250 MPa, there was a reduction of 50.1% compared with untreated samples. Pressures ranging from 200 to 250 MPa for 2 min were suitable to decrease weight loss in albacore without great impact in color and texture and, therefore, better retain the fresh quality.

4.2. Study 2: High-pressure pretreatment in albacore (*Thunnus alalunga*) for reducing freeze-driven weight losses with minimal quality changes

This work was focused on evaluating the impact of high-pressure processing (HPP) as a freezing pretreatment on quality parameters of albacore steaks after the freeze-thaw process. The HPP conditions employed were selected according with the results obtained in the previous work, where it was observed that HPP treatments ranging from 200 to 250 MPa for 2 min were suitable for decreasing weight loss of albacore with a minimal impact on fish quality.

Freezing has been commonly used for preservation of several fish species. However, negative physical and biochemical changes occur during the freeze–thaw process, such as lipid oxidation and protein denaturation (Gökoğlu & Yerlikaya, 2015b), as well as weight loss. Since weight loss involves economical and nutrient losses, it is an important issue for the seafood industry (Truong et al., 2016).

High-pressure processing (HPP) has shown potential to reduce thawing and cooking losses in fresh seafood (Shang et al., 2015; Xuan et al., 2018) due to structural changes in muscle proteins induced by the pressure, which can lead to protein gelation and better water retention (Chéret et al., 2005).

In recent years, HPP has been used as a pretreatment before freezing for reducing the negative effects of the freeze-thaw process on seafood quality (Aubourg, Torres, et al., 2013; Méndez et al., 2017; Vázquez et al., 2013), showing promising result, such as an inhibition of lipid hydrolysis development (Méndez et al., 2017) or an improved acceptance of HPP-pretreated samples (Aubourg, Torres, et al., 2013; Torres et al., 2014). In this work, HPP pretreatment before freezing is proposed for decreasing both thawing and cooking losses, without affecting the remaining fish quality parameters (color, texture, soluble protein, lipids oxidation).

4.2.1. Weight losses

Thawing losses (Figure 33a) of HPP-pretreated albacore at 200 MPa for 4–6 min, 250 MPa for 2–6 min and 300 MPa for 0–6 min, were significantly lower ($p \leq 0.05$) than in the control group, obtaining reductions ranging from 39.7% to 53.2%. A similar finding was obtained in HPP-pretreated pork meat at 600 MPa for 6 min before freezing after 0 weeks of frozen storage (Grossi et al., 2014). However, no effect of HPP pretreatment was found in barramundi after 0 weeks of frozen storage between 150 and 250 MPa, while a higher drip loss was found at 300 MPa in comparison with the non-treated samples

(Truong et al., 2016). These contradictory results could be due to the different methodology used to calculate thawing loss and the differences between fish species. Albacore muscle proteins are much more stable to denaturation, degradation, and coagulation than those of other fish species, resembling rabbit or beef meat (Venugopal, 2005a).

The effect of pressurization time was less pronounced as the pressure increased. At 200 MPa, thawing loss progressively decreased with increasing pressurization time. However, there was no effect ($p > 0.05$) of pressurization time on thawing loss of albacore in pretreated samples at 300 MPa, and also at 250 MPa for above 2 min. The increase in water retention is mainly caused by the pressure-induced changes in the myofibrillar proteins of fish muscle (Huff-Lonergan & Lonergan, 2005; Puértolas & Lavilla, 2020). At 250–300 MPa these proteins are normally completely denatured (Puértolas & Lavilla, 2020), so the effect of pressurization time at these or higher pressures is negligible.

The pressure only affected thawing loss ($p \leq 0.05$) of albacore steaks at pressurization times of 0 min, showing lower values at 300 MPa than at 200 and 250 MPa. Xuan et al. (2018) also observed a decreasing trend in drip loss of fresh razor clam after HPP treatment between 200 and 300 MPa for 1–10 min.

High-pressure processing pretreated samples at 200–250 MPa for 0–6 min and 300 MPa for 0 min showed a lower ($p \leq 0.05$) cooking loss than the control samples, representing reductions between 27.4% and 55.4% in comparison with the controls (Figure 33b). Similarly, Souza et al. (2011) observed a lower cooking loss in HPP-treated (215 MPa, 15 s) fresh pork *longissimus dorsi* muscle compared to non-treated samples. The effect of pressurization time on cooking loss was different depending on the pressure. At 200 MPa, cooking loss decreased as the pressurization time increased; at 250 MPa no changes were found, and at 300 MPa an increment in cooking loss was found as the pressurization time increased. Thus, intense HPP pretreatments would lead to increased cooking losses. For example, McArdle et al. (2010) found an increasing trend in cooking loss for fresh beef as the pressure increased (200–400 MPa for 10 min). This increase was attributed to sarcoplasmic protein denaturation due to pressure (McArdle et al., 2010).
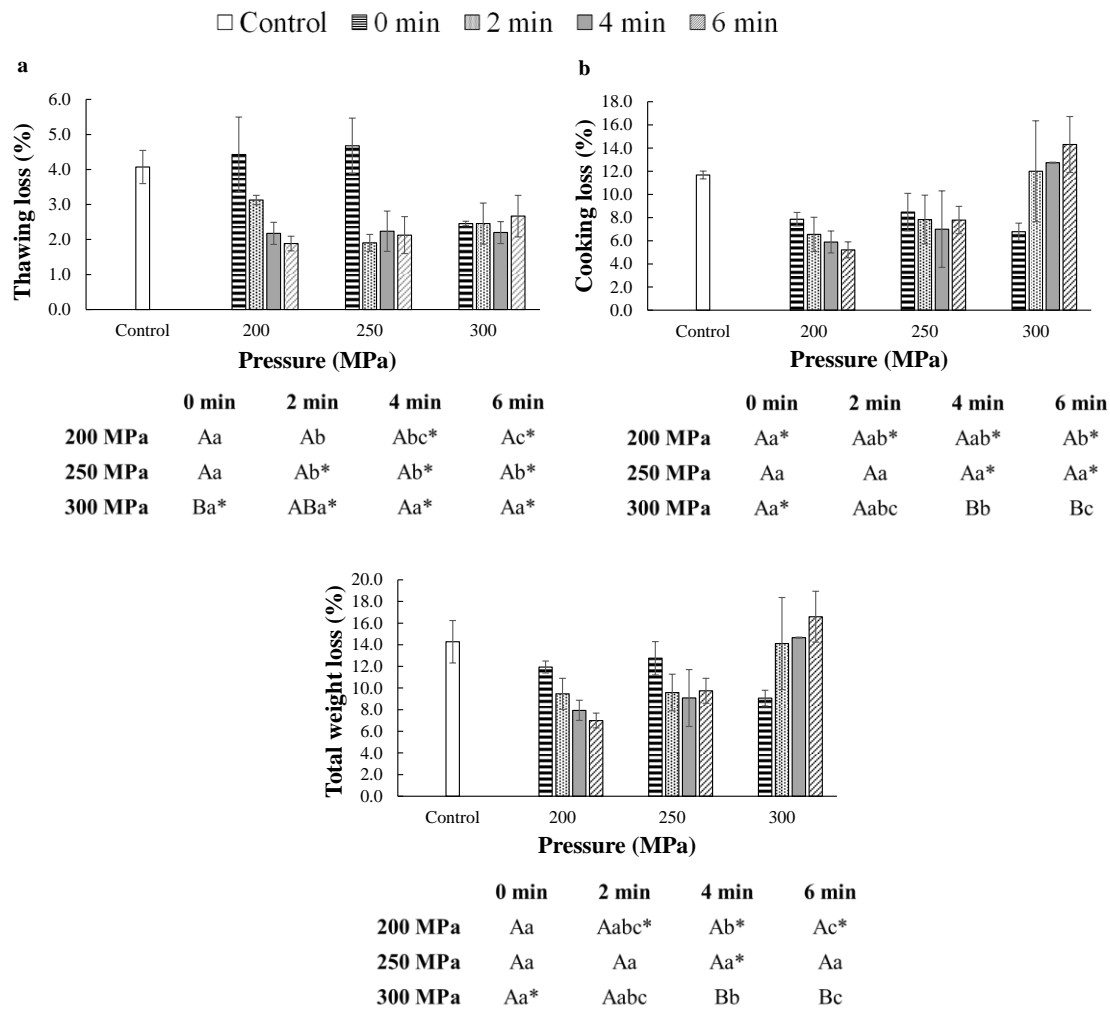
Figure 33. Thawing loss (a), cooking loss (b), and total weight loss (c) of albacore steaks thawed immediately after freezing previously treated by HPP (200–300 MPa/0–6 min). Values of control samples (0.1 MPa) were also shown for comparison. Mean values of three replicates; errors bars indicate 95% CI. For each pressurization time, different capital letters indicate significant differences ($p \leq 0.05$) as a result of the pressure applied. For each pressure, different lowercase letters indicate significant differences ($p \leq 0.05$) as a result of the pressurization time. An asterisk (*) indicates significant differences ($p \leq 0.05$) in comparison with the control (0.1 MPa) samples.

Total weight loss (Figure 33c) of HPP-pretreated albacore changed in the same manner as cooking loss. It could be explained because the cooking loss was higher than the thawing loss for all the samples. High-pressure processing pretreated samples treated at 200 and 250 MPa for above 2 min and 300 MPa for 0 min showed lower total weight loss than control samples, representing reductions between 31.7 and 51.0%.

The freeze-driven weight loss reductions observed after HPP pretreatment have been related to the protein structural changes induced by pressure (Puértolas & Lavilla, 2020). In this regard, several authors observed muscle compaction and protein gelation in micrographs of HPP-treated fish samples (Chéret et al., 2005; Truong et al., 2017; Xuan

et al., 2018), which could lead to the muscle displaying better water retention ability (Chéret et al., 2005).

4.2.2. Color

Excluding samples pretreated at 200 and 250 MPa for 0 min, the remaining HPP-pretreated samples showed higher L^* values ($p > 0.05$) than the controls (Table 2). Similar results were found in other fish species pretreated at similar HPP conditions at time 0 of frozen storage (Aubourg, Torres, et al., 2013; Pita-Calvo et al., 2018a; Ramirez-Suarez & Morrissey, 2006; Torres et al., 2014; Truong et al., 2016) and also in fresh samples (Cartagena et al., 2019; Chouhan et al., 2015). L^* value gradually increased with increasing pressurization time. However, at 300 MPa there were no differences ($p > 0.05$) between all the HPP-pretreated samples for above 2 min. A similar effect was found in minced albacore (275–310 MPa for 2–6 min) (Ramirez-Suarez & Morrissey, 2006). The increase in the L^* value has been attributed to globin and myofibrillar denaturation (Chouhan et al., 2015; Puértolas & Lavilla, 2020).

There were no differences ($p > 0.05$) in the a^* value between HPP-pretreated albacore steaks and the control group (Table 2), irrespective of the pressurization time or the pressure applied. These results are in accordance with those obtained in several HPP-pretreated (150–450 MPa for 0–5 min) fish species before freezing at time 0 of frozen storage, such as Atlantic mackerel (*Scomber scombrus*) or horse mackerel (*Trachurus trachurus*) (Aubourg, Torres, et al., 2013; Torres et al., 2014).

Only high-pressure processing pretreatments at 200 MPa for 0–2 min and 250 MPa for 0 min resulted in a similar b^* value ($p > 0.05$) to that found in the control samples (Table 2). As L^* value, b^* also increased with both pressure and pressurization time. Denaturation of myofibrillar and sarcoplasmic proteins (Chouhan et al., 2015) or to the development of lipid oxidation (Torres et al., 2014) have been related to changes in b^* value after HPP treatment.

Those HPP conditions that led to a greater decrease in exudate leak resulted in noticeable changes in L^*, b^* and ΔE of albacore steaks: 200 MPa for 2 min resulted in a noticeable decrease in exudate leaks without involving visually perceptible changes in albacore steaks (L^* and b^* value increased by 11.3% and 8.5% with respect to the control,

respectively). Although pressures below 200 MPa barely modified color parameters in fresh albacore, they led to higher weight losses than the controls (Cartagena et al., 2019).

Table 2. L^*, a^* value, b^* value and total color difference (ΔE) of albacore steaks thawed immediately after freezing previously treated by HPP (200–300 MPa/0–6 min).

| Time (min) | Control | HPP pretreatment | | |
| | | Pressure (MPa) | | |
		200	250	300
L*				
0	45.3 ± 1.8	46.1 ± 0.9^{Aa}	45.7 ± 0.8^{Aa}	$52.0 \pm 1.0^{Ba*}$
2		$50.5 \pm 1.4^{Ab*}$	$56.5 \pm 1.1^{Bb*}$	$59.5 \pm 0.9^{Cb*}$
4		$52.3 \pm 2.0^{Ab*}$	$60.1 \pm 1.4^{Bc*}$	$60.3 \pm 1.0^{Bb*}$
6		$56.3 \pm 1.8^{Ac*}$	$58.6 \pm 0.9^{Bbc*}$	$60.1 \pm 0.6^{Bb*}$
a*				
0	6.5 ± 1.2	5.6 ± 0.9^{Aa}	4.5 ± 1.6^{Aa}	5.3 ± 0.3^{Aa}
2		$5.1 \pm 0.9^{Aa*}$	$6.0 \pm 0.7^{Aa*}$	5.4 ± 0.2^{Aa}
4		$4.2 \pm 1.1^{Aa*}$	5.3 ± 0.9^{Aa}	5.6 ± 0.5^{Aa}
6		$5.6 \pm 1.2^{Aa*}$	6.2 ± 0.4^{Aa}	5.7 ± 0.5^{Aa}
b*				
0	4.3 ± 0.9	4.3 ± 0.6^{Aa}	4.3 ± 0.5^{Aa}	$6.9 \pm 0.6^{Aa*}$
2		4.7 ± 0.5^{Aab}	$9.4 \pm 0.3^{Bb*}$	$8.9 \pm 0.3^{Ab*}$
4		$5.8 \pm 0.4^{Ab*}$	$10.4 \pm 0.4^{Bc*}$	$9.3 \pm 0.4^{Bb*}$
6		$9.1 \pm 0.6^{Ac*}$	$9.9 \pm 0.5^{Abc*}$	$9.3 \pm 0.3^{Cb*}$
ΔE				
0	–	1.2 ± 0.6^{Aa}	2.0 ± 1.3^{Aa}	7.2 ± 1.1^{Ba}
2		5.3 ± 0.4^{Aa}	12.3 ± 1.1^{Bb}	15.0 ± 0.9^{Cb}
4		7.5 ± 2.2^{Ab}	16.0 ± 1.4^{Bc}	15.8 ± 0.9^{Bb}
6		12.0 ± 1.9^{Ac}	14.4 ± 0.9^{Bc}	15.6 ± 0.7^{Bb}

All values are means $\pm$ 95% CI. For each pressurization time, different capital letters indicate significant differences ($p \leq 0.05$) as a result of the pressure applied. For each pressure, different lowercase letters indicate significant differences ($p \leq 0.05$) as a result of the pressurization time. Asterisk (*) indicates significant differences ($p \leq 0.05$) in comparison with control (0.1 MPa) samples.

High-pressure processing pretreatment at 200 MPa for 6 min, 250 MPa for above 2 min, and 300 MPa for above 2 min resulted in a total color difference (ΔE) higher than 10 units (Table 2), which indicates visually perceptible changes (Jung et al., 2003). ΔE gradually increased with both the pressure and the pressurization time.

4.2.3. Texture

High-pressure processing pretreatments at 200 MPa for 4 min, 250 MPa for 6 min and 300 MPa for above 2 min resulted in a significantly greater hardness ($p \leq 0.05$) than the controls (Figure 34a). A similar behavior was observed in HPP-pretreated (275–310 MPa for 2–6 min) minced albacore before freezing (Ramirez-Suarez & Morrissey, 2006). Hardness gradually increased with both pressure and pressurization time. An increase in hardness due to the pressurization time was found at higher pressures (≥ 250 MPa). Similar findings were observed in HPP-pretreated (150–450 MPa for 0–5 min) Atlantic mackerel before freezing (Aubourg, Torres, et al., 2013). Regarding the pressure effect in hardness, an increasing trend was observed at pressurization times above 2 min. Protein denaturation induced by pressure and the formation of new hydrogen bonded networks (Angsupanich & Ledward, 1998; Kong et al., 2015) could be involved in hardness changes after HPP pretreatments.

Excluding 200 and 250 MPa for 0 min, all HPP pretreatments resulted in a markedly higher adhesiveness ($p \leq 0.05$) than the controls (Figure 34b). In a similar study, HPP-pretreated horse mackerel (150–450 MPa for 0–5 min) before freezing also showed an increment in adhesiveness due to HPP pretreatment (Torres et al., 2014). An increase in adhesiveness could be caused by the unfolding of actin, which would occur from 200 MPa, as well as the formation of new hydrogen bonded networks (Alves de Oliveira et al., 2017; Angsupanich & Ledward, 1998). At 200 and 250 MPa, adhesiveness gradually increased with the pressurization time, while at 300 MPa there were no changes ($p > 0.05$). Similar increasing adhesiveness trends have been highlighted in both HPP-pretreated Atlantic and horse mackerels (150–450 MPa for 0–5 min) (Aubourg, Torres, et al., 2013; Torres et al., 2014).

The springiness of all HPP-pretreated samples was significantly different ($p \leq 0.05$) from the controls (Figure 34c). The springiness increase has been related to the increase in hardness and the formation of hydrogen-bonded network induced by HPP pretreatment (Angsupanich & Ledward, 1998; Puértolas & Lavilla, 2020). At lower pressures (200 MPa), the effect of pressurization time on springiness was more important than at higher pressures (≥ 250 MPa) (Truong et al., 2016). These results are in accordance with those reported by Kaur et al. (2016), who did not observe changes in springiness of fresh black tiger shrimp (*Penaeus monodon*) due to the pressurization time after HPP treatments at 300 MPa for 3–15 min. In accordance with Truong et al. (2016), who did

not find any effect of pressure on springiness at 0 weeks of frozen storage in HPP-pretreated (150–300 MPa for 3 min) barramundi before freezing, a clear effect on springiness as a result of the pressure was not observed.
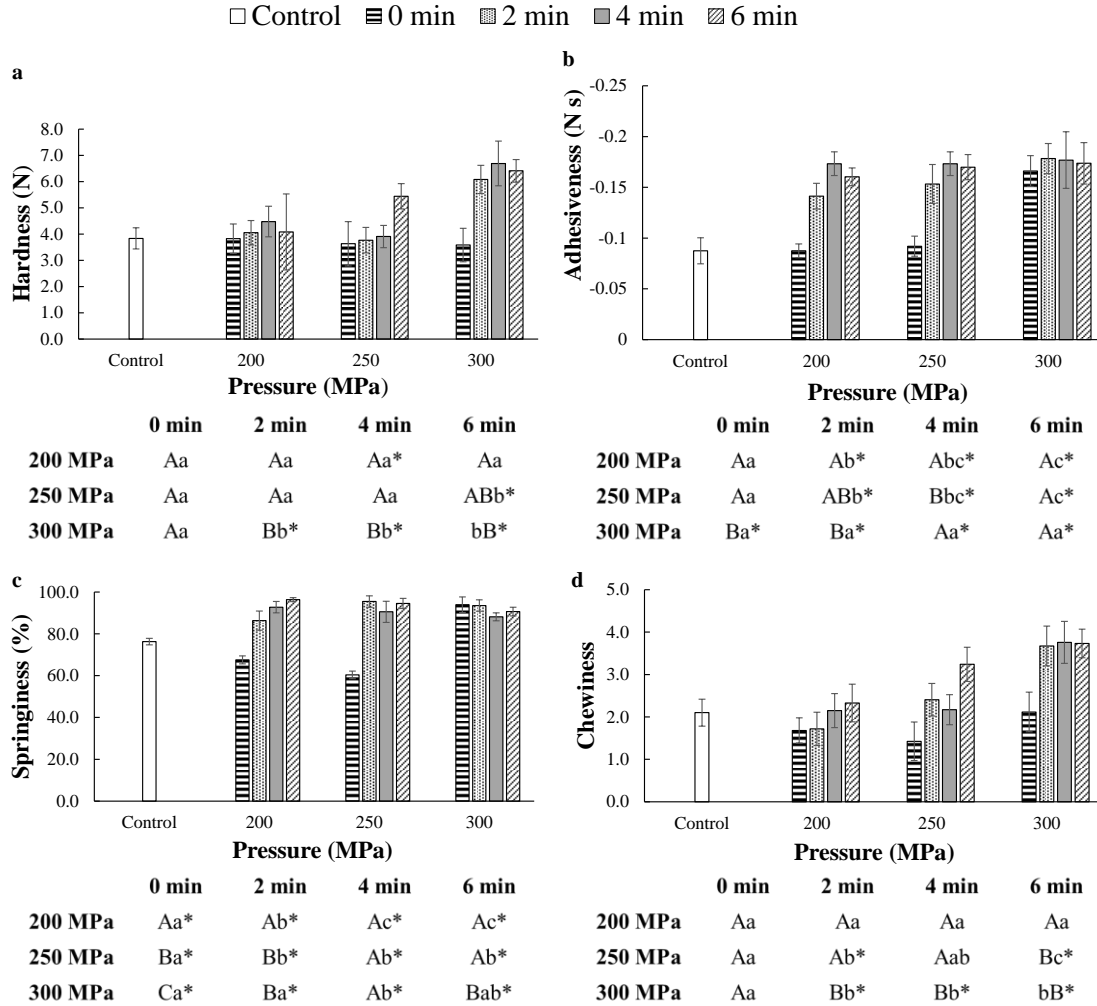
Figure 34. Hardness (a), adhesiveness (b), springiness (c), and chewiness (c) of albacore steaks thawed immediately after freezing previously treated by HPP (200–300 MPa/0–6 min). Values of control samples (0.1 MPa) were also shown for comparison. Mean values of three replicates; errors bars indicate 95% CI. For each pressurization time, different capital letters indicate significant differences ($p \leq 0.05$) as a result of the pressure applied. For each pressure, different lowercase letters indicate significant differences ($p \leq 0.05$) as a result of the pressurization time. An asterisk (*) indicates significant differences ($p \leq 0.05$) in comparison with control (0.1 MPa) samples.

No differences ($p > 0.05$) in chewiness were found in any of the HPP-pretreated samples at 200 MPa with respect to the controls (Figure 34d), 250 MPa for 2 and 6 min and 300 MPa for above 2 min resulted in a higher chewiness ($p \leq 0.05$) than the controls. From a general point of view, chewiness increased in albacore with both pressure and time. Similar behaviors have been published in other seafood species like Atlantic mackerel,

hake (*Merluccius merluccius*) and black tiger shrimp (Aubourg, Torres, et al., 2013; Kaur et al., 2016; Pita-Calvo et al., 2018a).

From a texture perspective, HPP pretreatments at 200 MPa for 2–6 min and 250 MPa for 2–4 min before freezing allowed to better retain the characteristic texture of albacore steaks. For instance, HPP-pretreated albacore at 200 MPa for 2 min showed a 5.6%, 61.5% and 13.2% increase in hardness, adhesiveness and springiness, respectively, and a 18.2% decrease in chewiness in comparison with the control. In HPP-pretreated albacore at 250 MPa for 4 min, hardness, adhesiveness, springiness, and chewiness increased by 1.8%, 98.1%, 18.7%, and 3.3%, respectively, with respect to the control. Similar HPP pretreatments (150–300 MPa for 0–5 min) in Atlantic mackerel also affected texture parameters in the same manner as in the present work, while it did not negatively affect the sensory properties (Aubourg, Torres, et al., 2013).

4.2.4. Salt-soluble protein content

Salt-soluble protein content indicates the degree of myofibrillar protein denaturation and aggregation (Truong et al., 2017). In general terms, HPP pretreatment decreased salt-soluble protein content in comparison with non-treated samples (Figure 35). Only HPP pretreatments at 200 MPa for 0 min showed similar values ($p > 0.05$) to the controls. At time 0 of frozen storage, similar HPP conditions (from 150 to 200 MPa for 0 min) in sardine (*Sardina pilchardus*) showed no significant differences in comparison to controls (Méndez et al., 2017). Hence, higher pressures than those tested by Méndez et al. (2017) could result in significantly lower salt-soluble protein content than the non-treated sardine. Salt-soluble protein content decreased as the pressure increased. In agreement, Méndez et al. (2017) reported an inverse correlation between myofibrillar content and pressure in high-pressure pretreated sardine before freezing.

In the albacore samples, the increase of pressurization time at 200 MPa decreased salt-soluble protein content. However, above 250 MPa no effect of pressurization time was observed.
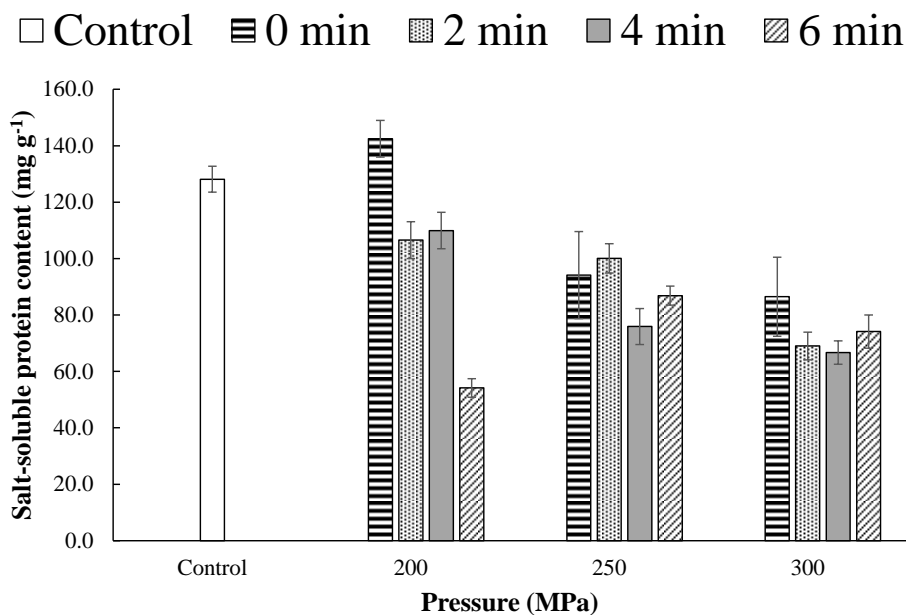
	0 min	**2 min**	**4 min**	**6 min**
200 MPa	Aa	Ab*	Ab*	Ac*
250 MPa	Bab*	Aa*	Bb*	Bab*
300 MPa	Ba*	Ba*	Ba*	Ca*

Figure 35. Salt-soluble protein content (mg g^{-1} muscle) of albacore steaks thawed immediately after freezing previously treated by HPP (200–300 MPa/0–6 min). Values of control samples (0.1 MPa) were also shown for comparison. All values are the mean of three replicates ± 95% CI. For each pressurization time, different capital letters indicate significant differences ($p \leq 0.05$) as a result of the pressure applied. For each pressure, different lowercase letters indicate significant differences ($p \leq 0.05$) as a result of the pressurization time. An asterisk (*) indicates significant differences ($p \leq 0.05$) in comparison with control (0.1 MPa) samples.

4.2.5. TBARS value

High-pressure pretreatment before freezing did not strongly change TBARS value in all HPP-pretreated samples with respect to the control samples (Table 3). Similar results were reported by Vázquez et al. (2013), who did not find a clear effect on TBARS value of HPP-pretreated (150–450 MPa for 0–5 min) Atlantic mackerel before freezing due to both pressurization time and pressure. As there were no changes in TBARS due to HPP pretreatment before freezing in albacore samples, the increase in b^* value detected in the present work could not be justified by lipid oxidation development (Torres et al., 2014), but by denaturation of myofibrillar and sarcoplasmic proteins (Chouhan et al., 2015). However, lipid oxidation compounds related to increase in b^* value could be Schiff bases and fluorescent compounds (Torres et al., 2014), which are not detected by TBARS index.

Table 3. TBARS value (mg MDA kg^{-1} muscle) of albacore steaks thawed immediately after freezing previously treated by HPP (200–300 MPa/0–6 min).

Time (min)	Control	Pressure (MPa)		
		200	250	300
0	1.08 ± 0.04	1.02 ± 0.07^{Aab}	0.98 ± 0.10^{Aa}	1.20 ± 0.14^{Aa}
2		0.82 ± 0.03^{Aa}	0.91 ± 0.01^{Aba}	1.01 ± 0.07^{Ba}
4		1.07 ± 0.11^{Ab}	0.97 ± 0.18^{Aa}	1.03 ± 0.06^{Aa}
6		1.07 ± 0.03^{Ab}	1.14 ± 0.26^{Aa}	0.99 ± 0.08^{Aa}

All values are means ± 95% CI. For each pressurization time, different capital letters indicate significant differences ($p \leq 0.05$) as a result of the pressure applied. For each pressure, different lowercase letters indicate significant differences ($p \leq 0.05$) as a result of the pressurization time. Asterisk (*) indicates significant differences ($p \leq 0.05$) in comparison with control (0.1 MPa) samples.

From these results it can be concluded that HPP pretreatment led to a reduction in exudate leaks due to the freeze–thaw process when processing conditions (pressure and pressurization time) were properly selected. At lower pressures (200 MPa), there was a clear effect of pressurization time on most fish quality parameters tested (thawing loss, $L*$ value, $b*$ value, ΔE, adhesiveness, springiness, salt-soluble protein content), whereas at higher pressures (300 MPa), similar changes took place independently of the pressurization time: 200 MPa for 6 min allowed the maximum reduction of both thawing and cooking losses (a 53.7% and 55.4% decrease compared to control samples, respectively) to be achieved, involving a total weight loss 51.0% lower than the controls. However, this HPP pretreatment caused noticeable changes in color (a 24.2% and 111.6% increase in $L*$ and $b*$ value with respect to controls, respectively; ΔE higher than 10 units), which could strongly affect consumer acceptance. At 200 MPa for 2 min there were also important reductions in thawing, cooking, and total losses with respect to controls (decreases of 23.2% and 44.0% and 33.7%, respectively). However, this treatment did not have a great impact on color (a 11.3% and 8.5% increase in $L*$ and $b*$ value, respectively; ΔE round 5 units). Therefore, 200 MPa for 2 min could be a good compromise treatment, allowing thawing and cooking losses to be reduced in albacore, decreasing HPP-mediated color alterations and the related possible consumer rejection.

4.3. Study 3: Evolution of quality parameters of high-pressure processing (HPP) pretreated albacore (*Thunnus alalunga*) during long-term frozen storage

This chapter evaluated the potential benefits of high-pressure processing (HPP) pretreatment for improving quality of albacore after long term-storage.

Despite the fact that albacore (*Thunnus alalunga*) is a highly demanded fish, its market availability is limited due to its high seasonality and the fishing quotes that regulate the annual quantity caught by European fisheries (Pusineri et al., 2005). Frozen storage could ensure albacore market availability throughout the year.

Previous results showed that HPP pretreatments before freezing allowed the reduction of both thawing and cooking losses of frozen albacore after the freeze-thaw process. However, the fish quality can also change during the frozen storage (Ben-Gigirey et al., 1999; Leygonie et al., 2012). Hence, in this work HPP pretreatment before freezing is proposed for improving the quality of albacore steaks after long-term frozen storage.

Since albacore is normally consumed after a thermal treatment (once cooked or canned) the effect of HPP pretreatment before freezing on quality parameters was evaluated in both raw and cooked samples.

4.3.1. Thawing, cooking and total weight losses

Table 4 shows thawing loss (a), cooking loss (b) and total weight loss (c) of HPP-pretreated albacore before freezing and frozen storage. At time 0 of frozen storage, thawing loss of control samples was similar to those obtained in bigeye tuna samples after one freeze-thaw cycle (Q. Jiang et al., 2019). Thawing loss increased during the first 2 months of frozen storage independently of the treatment conditions. However, it remained stable or slightly decreased after 2 months and up to 12 months of frozen storage.

After 12 months of storage, control frozen samples presented a 55.0% higher thawing loss ($p \leq 0.05$) than the fresh (non-frozen) control samples. However, there were no differences ($p > 0.05$) between HPP-pretreated samples at 200 MPa for 4–6 min after 12 months of frozen storage and fresh (non-frozen) control samples. Therefore, HPP pretreatment before freezing could minimize thawing loss after long-term storage. Reduction in thawing losses was also reported in HPP-pretreated barramundi after 18 weeks of frozen storage (Truong et al., 2016). On the contrary, Yi et al. (2013) observed a higher thawing loss in HPP-pretreated bay scallop (*Argopecten irradians*) (200 MPa for 3 min, 350 MPa for 0 min) than in non-treated samples during up to 150 days of frozen storage. Although barramundi and bay scallop are very different in muscle characteristics,

in both cases changes in thawing loss after HPP treatment have been attributed to pressure-induced denaturation. According to that, HPP conditions must be optimized for reducing drip losses depending on the fish species. Below a pressure/time threshold, HPP results in a cell permeabilization increase, which led to the release of intracellular liquid, and thus, to an increase in fluid leaks. However, above this pressure/time threshold, changes in protein structure induced by HPP, which improves water retention, compensate the induced permeabilization, leading to a minor fluid leak (Cartagena et al., 2019).

In thawed samples just after freezing (0 months), pretreatments at 200 MPa for 4 and 6 min allowed the reduction of cooking loss ($p \leq 0.05$) with respect to the controls in a 44.8 and 46.3%, respectively (Table 4). However, this reduction disappeared just after 2 months of frozen storage. Thus, the positive effect of HPP pretreatment on thawing loss during frozen storage was not observed in cooking loss.

It has been suggested that fluid leak reduction after HPP treatment is caused by pressure-induced gelation of proteins (Chéret et al., 2005), consisting of hydrogen-bonded networks, while cooking and thermal treatments cause different protein changes, which result in a minor ability to retain water (Chouhan et al., 2015; Tornberg, 2005). Current results on thawing and cooking losses showed that the negative effect of the cooking process on water retention has a stronger impact than the positive effect of HPP.

The total weight loss of the albacore samples after freezing, thawing and cooking was also calculated (Table 4). In thawed samples just after freezing (0 months), pretreatments of 200 MPa for 4 and 6 min reduced ($p \leq 0.05$) total weight loss with respect to the controls in a 38.8 and a 43.4%, respectively. Pretreated albacore at 200 MPa for 6 min presented also a similar reduction ($p \leq 0.05$) during the first 6 months of frozen storage. However, after 12 months no differences ($p > 0.05$) were observed in total weight loss between any HPP-pretreated samples and the controls.

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Table 4. Thawing loss, cooking loss and total weight loss after HPP treatments (200 MPa for 0−6 min) before freezing and frozen storage time (up to 12 months). Fresh albacore steaks were also included for comparison.

	Control	Pressure/time (MPa/min)			
		200/0	200/2	200/4	200/6
Thawing loss (%)					
Fresh	2.74 ± 0.30^{Aa}	4.31 ± 0.24^{Ab}	2.74 ± 0.30^{Aa}	2.96 ± 0.46^{Aa}	1.74 ± 0.23^{Ac}
0	3.84 ± 0.40^{Ba}	5.01 ± 0.46^{Ab}	4.06 ± 0.43^{Ba}	2.86 ± 0.48^{Ac}	2.68 ± 0.51^{ABc}
2	4.63 ± 0.59^{Bab}	6.33 ± 0.69^{Bc}	5.65 ± 0.71^{Cbc}	4.10 ± 0.43^{Bad}	3.26 ± 0.50^{Bd}
6	4.04 ± 0.37^{Bab}	5.24 ± 0.38^{ABc}	4.73 ± 0.50^{BCbc}	3.70 ± 0.24^{ABa}	2.69 ± 0.33^{ABd}
12	4.25 ± 0.35^{Ba}	4.48 ± 0.91^{Aa}	4.06 ± 0.36^{Ba}	2.81 ± 0.50^{Ab}	2.46 ± 0.14^{ABb}
Cooking loss (%)					
Fresh	11.69 ± 1.86^{Aa}	10.83 ± 1.69^{Aabc}	11.38 ± 1.27^{Aab}	8.15 ± 0.66^{ABc}	8.64 ± 1.16^{ABbc}
0	7.93 ± 2.35^{Ba}	8.33 ± 1.10^{Aba}	8.15 ± 1.60^{Ba}	6.57 ± 0.68^{Aa}	6.33 ± 1.52^{Aa}
2	8.52 ± 1.62^{ABa}	7.64 ± 0.82^{Ba}	9.65 ± 0.98^{Aba}	8.62 ± 0.57^{Ba}	8.24 ± 0.50^{ABa}
6	8.86 ± 0.62^{ABab}	8.86 ± 1.07^{ABab}	9.94 ± 0.99^{Aba}	9.21 ± 0.73^{Bab}	7.92 ± 1.32^{ABb}
12	6.63 ± 0.95^{Ba}	6.83 ± 0.93^{Bab}	8.54 ± 0.47^{Bbc}	9.22 ± 0.59^{Bc}	8.77 ± 0.70^{Bc}
Total weight loss (%)					
Fresh	14.06 ± 1.69^{Aa}	14.67 ± 1.60^{Aa}	13.83 ± 1.30^{ABa}	10.68 ± 0.84^{ABCb}	10.22 ± 1.21^{ABb}
0	11.48 ± 2.38^{ABab}	12.54 ± 1.09^{ABb}	11.67 ± 2.07^{Aab}	8.95 ± 0.85^{Aa}	8.27 ± 1.67^{Aa}
2	13.08 ± 1.29^{ABab}	13.64 ± 1.03^{ABab}	14.61 ± 0.67^{Bb}	12.58 ± 0.43^{BCbc}	11.25 ± 0.70^{Bc}
6	12.62 ± 0.75^{ABa}	13.80 ± 1.30^{ABa}	14.55 ± 1.02^{ABa}	12.82 ± 0.87^{Ca}	9.89 ± 1.41^{ABb}
12	10.14 ± 0.59^{Ba}	11.06 ± 1.19^{Ba}	12.40 ± 0.60^{ABa}	11.61 ± 0.58^{ABa}	10.84 ± 0.87^{Ba}

Mean $\pm$ 95% CI. For each frozen storage time, different lowercase letters indicate significant differences ($p \leq 0.05$) as a result of the HPP pretreatment. For each HPP pretreatment, different capital letters indicate significant differences ($p \leq 0.05$) as a result of frozen storage time.

In conclusion, an HPP pretreatment of 200 MPa for 4 or 6 min would allow the reduction of thawing loss of albacore steaks during up to 12 months of frozen storage. Although HPP pretreatment did not impact on cooking loss, the total weight loss was also reduced at 200 MPa for 6 min for up to 6 months of frozen storage

4.3.2. Color

Table 5 shows L^*, a^* and b^* values and total color difference (ΔE) of both raw and cooked samples of HPP-pretreated albacore before freezing (fresh) and during frozen storage (0–12 months). In raw samples, L^* and b^* values gradually increased due to HPP pretreatment in both fresh and frozen albacore, showing the highest values at 200 MPa for 6 min, independently of the frozen storage time. Similar results were obtained by Ramirez-Suarez & Morrissey (2006) in fresh and frozen minced albacore. L^* values obtained in fresh control were similar to those previously found by Cartagena et al. (2019).

Frozen storage did not impact on L^* of raw samples, independently of the treatment conditions (Table 5). Similar findings were reported in HPP-pretreated barramundi and hake (Pita-Calvo et al., 2018b; Truong et al., 2016). By contrast, an increasing trend in L^* during frozen storage was reported in Atlantic and horse mackerels (Aubourg, Torres, et al., 2013; Torres et al., 2014). b^* value of albacore steaks increased with the frozen storage in both controls and HPP-pretreated samples. The same effect was reported in hake during up to 9 months of frozen storage (Pita-Calvo et al., 2018b). Chouhan et al. (2015) suggested that it could be caused by denaturation of myofibrillar and sarcoplasmic proteins.

Comparing with the controls, HPP pretreatment did not affect the a^* value of raw albacore steaks independently of the frozen storage time (Table 5). Similar results were found in other HPP-pretreated fish species, such as horse mackerel, Atlantic mackerel or hake (Aubourg, Torres, et al., 2013; Pita-Calvo et al., 2018b; Torres et al., 2014).

There were no changes ($p > 0.05$) in a^* value of raw albacore steaks during the first 6 months of frozen storage, although a marked increase was observed from 6 months ($p \leq 0.05$).

Table 5. L^*, a^* value, b^* value and total color difference (ΔE) of both raw and cooked albacore steaks after HPP treatments (200 MPa for 0−6 min) before freezing and during frozen storage time (up to 12 months). Fresh albacore steaks were also included for comparison.

Frozen storage time (months)	Raw albacore					Cooked albacore				
	Control	Pressure/time (MPa/min)				Control	Pressure/time (MPa/min)			
		200/0	200/2	200/4	200/6		200/0	200/2	200/4	200/6
L^*										
Fresh	40.18 ± 0.74^{ABa}	43.28 ± 0.68^{ABb}	47.79 ± 0.58^{ABc}	52.79 ± 0.89^{Bd}	55.24 ± 1.48^{Ce}	72.38 ± 2.35^{Bab}	70.12 ± 0.87^{Bab}	69.59 ± 2.27^{Aa}	70.81 ± 1.56^{Bab}	73.42 ± 0.88^{Bb}
0	41.02 ± 0.90^{Ba}	42.97 ± 0.81^{ABb}	47.11 ± 0.99^{ABc}	49.85 ± 0.96^{Ad}	52.71 ± 1.14^{ABCe}	71.11 ± 1.79^{ABa}	69.30 ± 1.83^{ABa}	71.33 ± 1.31^{Aa}	69.80 ± 1.37^{Ba}	71.19 ± 1.43^{ABa}
2	39.94 ± 0.62^{ABa}	44.06 ± 0.73^{Bb}	47.87 ± 0.65^{ABc}	50.47 ± 1.00^{Ad}	52.62 ± 0.85^{ABCe}	68.03 ± 1.36^{ABa}	67.55 ± 1.13^{ABa}	68.12 ± 1.12^{Aa}	67.87 ± 1.77^{ABa}	68.63 ± 1.65^{ABa}
6	41.54 ± 0.68^{Ba}	44.66 ± 0.79^{Bb}	48.27 ± 0.74^{Bc}	50.87 ± 0.74^{ABd}	54.73 ± 0.84^{Be}	69.25 ± 1.64^{ABa}	69.67 ± 1.36^{Bab}	68.20 ± 1.50^{Aa}	67.82 ± 1.64^{ABa}	72.47 ± 1.61^{ABb}
12	38.63 ± 1.22^{Aa}	42.15 ± 1.76^{Ab}	46.24 ± 1.97^{Ac}	49.50 ± 0.98^{Acd}	51.60 ± 1.95^{Ad}	66.37 ± 5.74^{Aa}	66.23 ± 2.61^{Aa}	68.59 ± 2.30^{Aa}	64.21 ± 3.67^{Aa}	67.80 ± 3.26^{Aa}
a^*										
Fresh	3.13 ± 0.32^{Ab}	2.52 ± 0.36^{Aab}	2.00 ± 0.51^{Aa}	2.79 ± 0.56^{Aab}	3.39 ± 0.76^{Ab}	3.50 ± 0.72^{Aa}	3.92 ± 0.53^{Aa}	4.05 ± 0.91^{Aa}	3.75 ± 0.77^{Aa}	4.18 ± 0.74^{Aa}
0	3.20 ± 0.51^{Aab}	3.26 ± 0.61^{Aab}	2.20 ± 0.63^{Aa}	2.78 ± 0.62^{Aab}	3.95 ± 0.75^{Ab}	4.37 ± 0.68^{ABa}	4.05 ± 0.64^{Aa}	3.93 ± 0.53^{Aa}	4.45 ± 0.74^{ABa}	4.22 ± 0.55^{Aa}
2	3.63 ± 0.48^{Abc}	2.79 ± 0.39^{Aab}	2.18 ± 0.42^{Aa}	3.35 ± 0.62^{Abc}	3.94 ± 0.52^{Ac}	5.61 ± 0.64^{Ba}	4.63 ± 0.49^{Aa}	5.21 ± 0.59^{Aa}	5.29 ± 0.67^{ABa}	4.98 ± 0.29^{Aba}
6	3.66 ± 0.79^{Aab}	2.93 ± 0.45^{Aab}	2.54 ± 0.50^{Aa}	3.45 ± 0.48^{Aab}	3.67 ± 0.77^{Ab}	4.96 ± 0.73^{ABab}	4.07 ± 0.57^{Aa}	5.03 ± 0.67^{Aab}	5.66 ± 0.63^{Bb}	4.20 ± 0.75^{Aa}
12	5.11 ± 1.24^{Bab}	4.75 ± 0.85^{Bab}	4.33 ± 0.89^{Ba}	5.67 ± 0.67^{Bab}	6.25 ± 0.65^{Bb}	5.09 ± 1.95^{ABa}	5.91 ± 0.87^{Ba}	5.05 ± 1.25^{Aa}	4.60 ± 1.15^{ABa}	6.40 ± 0.62^{Ba}

b*

Fresh	0.64 ± 0.37^{Aa}	1.64 ± 0.36^{Aa}	2.95 ± 0.52^{Ab}	4.41 ± 0.61^{Ac}	5.90 ± 0.63^{Ad}	13.06 ± 0.56^{Aab}	13.17 ± 0.41^{ABb}	12.24 ± 0.47^{Aab}	12.11 ± 0.63^{Aa}	12.45 ± 0.41^{Aab}
0	2.79 ± 0.54^{Ba}	3.82 ± 0.51^{Ba}	5.24 ± 0.50^{Bb}	5.97 ± 0.58^{Bb}	7.96 ± 0.52^{BCc}	13.68 ± 0.45^{Ab}	14.07 ± 0.39^{Bb}	12.47 ± 0.39^{ABa}	13.30 ± 0.57^{ABab}	13.62 ± 0.25^{Ab}
2	3.29 ± 0.34^{Ba}	3.86 ± 0.32^{Ba}	5.37 ± 0.36^{Bb}	6.47 ± 0.54^{BCc}	6.82 ± 0.49^{ABc}	13.22 ± 0.69^{Aa}	12.82 ± 0.56^{Aa}	12.30 ± 0.77^{Aa}	12.83 ± 0.50^{ABa}	12.44 ± 0.63^{Aa}
6	4.87 ± 0.33^{Ca}	4.73 ± 0.31^{Ca}	6.02 ± 0.43^{BCb}	6.47 ± 0.36^{BCb}	7.98 ± 0.49^{BCc}	15.51 ± 0.65^{Ba}	13.55 ± 0.47^{ABb}	13.55 ± 0.38^{ABb}	13.67 ± 0.44^{Bb}	13.77 ± 0.50^{Ab}
12	4.70 ± 0.66^{Ca}	4.31 ± 0.56^{BCa}	7.00 ± 0.69^{Cb}	7.21 ± 0.64^{Cb}	8.11 ± 0.92^{Cb}	13.26 ± 2.20^{Aa}	13.88 ± 0.51^{Ba}	13.77 ± 1.21^{Ba}	13.29 ± 0.56^{ABa}	15.54 ± 2.00^{Ba}

ΔE

Fresh	–	3.33 ± 0.62^{Aa}	8.04 ± 0.54^{Ab}	13.16 ± 0.93^{Ac}	15.95 ± 0.91^{Ad}	–	2.30 ± 0.65^{Aa}	2.96 ± 0.49^{Aa}	1.85 ± 0.55^{Aa}	1.38 ± 0.68^{Aa}
0	–	2.20 ± 0.56^{Ba}	6.64 ± 0.73^{Ab}	9.39 ± 1.11^{BCc}	12.80 ± 0.96^{Bd}	–	1.88 ± 0.98^{Aa}	1.31 ± 0.81^{ABa}	1.37 ± 0.71^{Aa}	0.18 ± 0.89^{Aa}
2	–	4.25 ± 0.65^{ABa}	8.30 ± 0.70^{Ab}	11.01 ± 0.72^{ABc}	13.17 ± 0.80^{Bd}	–	1.16 ± 0.70^{Aa}	1.01 ± 0.60^{ABa}	0.54 ± 0.83^{Aa}	1.17 ± 0.78^{Aa}
6	–	3.15 ± 0.55^{ABa}	6.90 ± 0.64^{Ab}	9.47 ± 0.73^{Cc}	13.56 ± 0.71^{Bd}	–	2.22 ± 0.61^{Aa}	2.23 ± 0.90^{Ba}	2.43 ± 0.85^{Aa}	3.74 ± 0.73^{Aa}
12	–	3.63 ± 1.56^{ABa}	8.07 ± 1.41^{Ab}	11.16 ± 0.95^{ABCbc}	13.44 ± 1.86^{Bc}	–	1.03 ± 1.03^{Aa}	2.27 ± 0.37^{Ba}	2.22 ± 0.78^{Aa}	3.00 ± 0.72^{Aa}

Mean values ± 95% CI. For each frozen storage time, different lowercase letters indicate significant differences ($p \leq 0.05$) as a result of the HPP pretreatment. For each HPP pretreatment, different capital letters indicate significant differences ($p \leq 0.05$) as a result of frozen storage time.

ΔE of raw HPP-pretreated albacore steaks increased due to HPP pretreatment for any storage time, showing higher values than 10 units above 200 MPa for 4 min, which are considered to significantly modify the color appearance of meat (Jung et al., 2003) (Table 5). Similar findings were found in fresh skipjack tuna (*Katsuwonus pelamis*) after 1 day of frozen storage (Jiranuntakul et al., 2018).
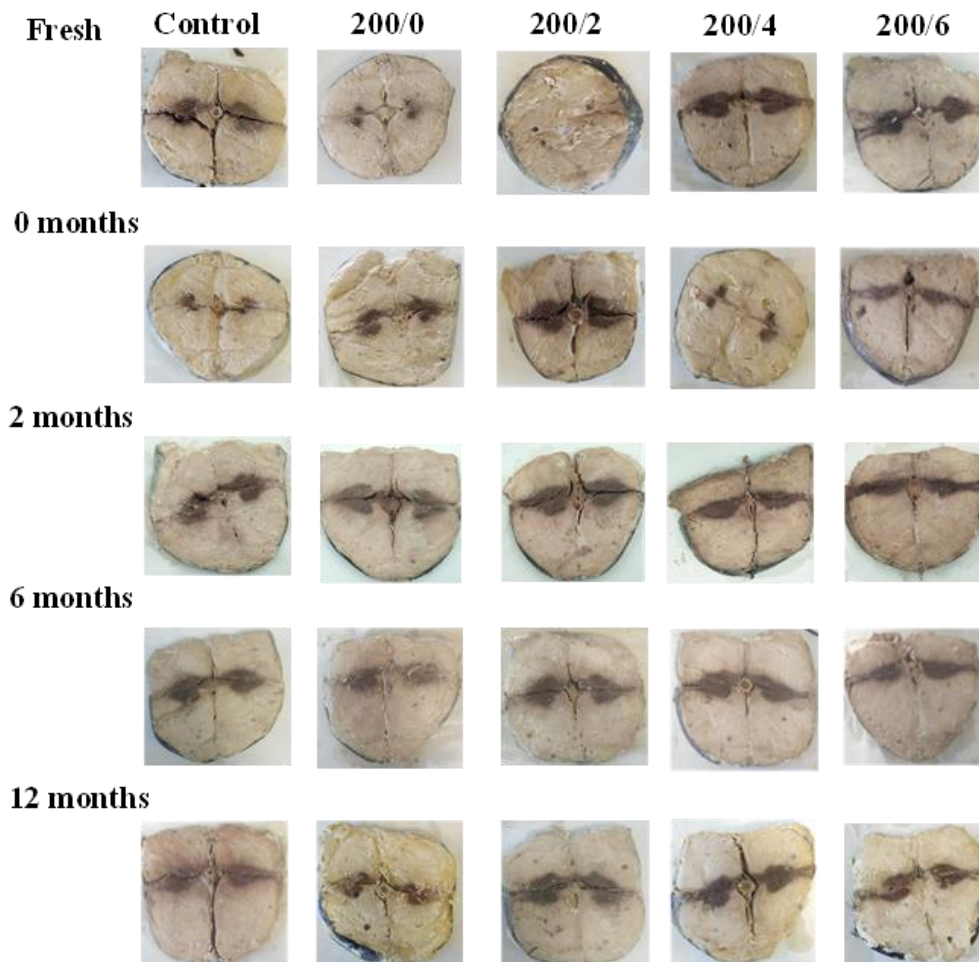
Figure 36. Appearance of cooked albacore steaks after HPP treatments (200 MPa for 0–6 min) before freezing and frozen storage time (up to 12 months). Fresh albacore steaks were also included for comparison.

After cooking, there was no effect ($p \leq 0.05$) of HPP pretreatment on L^*, a^*, b^* and ΔE values for any storage time (Table 5). Therefore, the increase in L^*, b^* and ΔE observed in raw albacore steaks due to HPP pretreatment did not occur upon cooking. Furthermore, all cooked samples resulted in higher L^* and b^* values than raw samples regardless of both HPP pretreatment and storage time. Thus, the impact of cooking on color of albacore steaks was stronger than the impact of HPP pretreatment. Furthermore, all cooked samples showed lower ΔE values than 10 units independently of HPP pretreatment or

frozen storage time. In terms of the visual appearance of cooked albacore steaks, no appreciable differences were found between samples regardless of HPP pretreatment (Figure 36).

Pressure and heat resulted in similar changes in color (increase of L^* and b^*), which may be caused by protein denaturation. Whereas HPP pretreatment led to denaturation of myofibrillar and sarcoplasmic proteins, cooking led to denaturation of hemoglobin and myoglobin (Chouhan et al., 2015).

4.3.3. Texture

Table 6 shows hardness, adhesiveness, springiness and chewiness of both raw and cooked HPP-pretreated albacore before freezing (fresh) and during frozen storage. Hardness values of HPP-pretreated raw albacore ranged between 3.5 and 5.5 N. Similar values were obtained in a previous work in the same fish species (Cartagena et al., 2019). At time 0 of frozen storage, hardness decreased for any HPP treatment in comparison to fresh samples. Thus, the freeze-thaw process could result in a hardness reduction, as observed Aubourg, Torres, et al. (2013), likely due to the ice crystal formation inherent to freezing, which lead to myofibrils disruption (Leygonie et al., 2012). Hardness of albacore increased throughout the frozen storage time. Similar results were also reported in other HPP-pretreated oily fish species, such as Atlantic and horse mackerels (Aubourg, Torres, et al., 2013; Torres et al., 2014). Rodríguez et al. (2015) also reported an increasing trend of hardness in Coho salmon (*Oncorhynchus kisutch*) during frozen storage (up to 18 months). These authors suggested that cross-linking of peptide chains with lipid oxidation compounds during frozen storage results in a higher hardness.

In raw albacore, hardness of fresh samples and those thawed just after freezing (time 0 of frozen storage) progressively increased with the pressure time. The same behavior was also observed in minced albacore (Ramirez-Suarez & Morrissey, 2006). Therefore, the effect of pressure on hardness would not be modified due to the freezing process.

When samples were stored (2–12 months), no differences were detected ($p \leq 0.05$) between HPP-pretreated samples and controls. Thus, frozen storage led to the vanishing of the initial increase of hardness caused by HPP pretreatment.

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Table 6. Hardness, adhesiveness, springiness and chewiness of raw and cooked albacore steaks after HPP treatments (200 MPa for 0−6 min) before freezing and frozen storage time (up to 12 months). Fresh albacore steaks were also included for comparison.

Frozen storage time (months)	Raw albacore					Cooked albacore				
		Pressure/time (MPa/min)					Pressure/time (MPa/min)			
	Control	200/0	200/2	200/4	200/6	Control	200/0	200/2	200/4	200/6
Hardness (N)										
Fresh	4.52 ± 0.49^{Ba}	4.38 ± 0.46^{ABa}	4.61 ± 0.39^{BCab}	4.99 ± 0.38^{ABab}	5.47 ± 0.54^{Ab}	12.68 ± 0.74^{Ac}	8.60 ± 0.62^{Aa}	9.58 ± 1.20^{Bab}	11.18 ± 0.95^{ABbc}	10.41 ± 1.09^{Aab}
0	3.49 ± 0.28^{Aa}	3.91 ± 0.36^{Aab}	3.76 ± 0.23^{Aab}	4.34 ± 0.37^{ABb}	4.27 ± 0.55^{Bb}	18.15 ± 1.26^{Ba}	18.06 ± 2.18^{Ca}	11.79 ± 1.32^{ABb}	9.23 ± 1.04^{ABb}	10.95 ± 1.06^{Ab}
2	4.33 ± 0.26^{Ba}	4.04 ± 0.26^{Aa}	3.93 ± 0.44^{ABa}	4.19 ± 0.33^{Aa}	4.09 ± 0.27^{Ba}	17.91 ± 1.35^{Ba}	18.74 ± 1.36^{Ca}	14.13 ± 1.32^{Ab}	11.48 ± 1.04^{Bc}	9.25 ± 1.12^{Ac}
6	4.55 ± 0.31^{Ba}	4.27 ± 0.27^{ABab}	4.56 ± 0.25^{BCa}	4.28 ± 0.35^{Aab}	3.89 ± 0.24^{Bb}	16.26 ± 1.36^{Ba}	16.73 ± 1.04^{Cab}	10.30 ± 0.99^{Bb}	9.09 ± 0.52^{Ab}	9.09 ± 0.68^{Ab}
12	5.02 ± 0.18^{Ba}	4.99 ± 0.27^{Ba}	5.30 ± 0.31^{Ca}	5.15 ± 0.26^{Ba}	4.68 ± 0.56^{ABa}	15.81 ± 1.60^{Ba}	13.08 ± 2.32^{Bab}	10.37 ± 1.00^{Bb}	9.68 ± 2.98^{ABb}	5.88 ± 0.95^{Bc}
Adhesiveness (N s)										
Fresh	0.129 ± 0.011^{Ab}	0.101 ± 0.012^{Ac}	0.120 ± 0.010^{Abc}	0.158 ± 0.015^{Aa}	0.175 ± 0.012^{ABCa}	0.020 ± 0.0056^{ABa}	0.020 ± 0.0048^{ABa}	0.031 ± 0.0073^{Ba}	0.031 ± 0.0075^{Ba}	0.032 ± 0.0076^{BCa}
0	0.097 ± 0.009^{Bbc}	0.083 ± 0.007^{Bc}	0.115 ± 0.011^{Ab}	0.157 ± 0.010^{Aa}	0.158 ± 0.010^{Ca}	0.016 ± 0.0043^{ABb}	0.023 ± 0.0056^{ABab}	0.022 ± 0.0049^{Bab}	0.033 ± 0.0074^{Ba}	0.034 ± 0.0072^{BCa}
2	0.085 ± 0.006^{BCbc}	0.077 ± 0.008^{Bc}	0.100 ± 0.015^{ABb}	0.149 ± 0.013^{Aa}	0.166 ± 0.010^{BCa}	0.013 ± 0.0036^{Bb}	0.018 ± 0.0055^{ABa}	0.030 ± 0.0062^{Ba}	0.024 ± 0.0058^{Bab}	0.030 ± 0.0056^{Ca}
6	0.085 ± 0.007^{BCa}	0.087 ± 0.006^{ABa}	0.120 ± 0.008^{Ab}	0.159 ± 0.015^{Ac}	0.183 ± 0.009^{ABd}	0.016 ± 0.0051^{ABc}	0.014 ± 0.0033^{Ac}	0.032 ± 0.0055^{Bb}	0.054 ± 0.0084^{Aa}	0.053 ± 0.0094^{ABa}
12	0.074 ± 0.006^{Cc}	0.074 ± 0.004^{Bc}	0.088 ± 0.004^{Bc}	0.140 ± 0.009^{Ab}	0.192 ± 0.013^{Aa}	0.026 ± 0.0048^{Ab}	0.030 ± 0.0186^{Bb}	0.056 ± 0.0165^{Aab}	0.069 ± 0.0145^{Aa}	0.070 ± 0.0239^{Aa}

Springiness (%)

Fresh	78.15 ± 2.94^Aa	76.57 ± 2.04^Ba	86.93 ± 2.71^Cb	90.88 ± 2.53^ABb	95.94 ± 1.04^ABc	67.60 ± 2.62^Aa	69.44 ± 2.40^Aa	69.56 ± 2.12^Ba	69.03 ± 4.45^Ca	66.50 ± 3.49^Ba
0	75.99 ± 1.97^Aa	70.63 ± 1.81^Ab	84.13 ± 3.18^BCc	91.41 ± 2.01^Bd	95.90 ± 0.92^ABe	65.95 ± 2.69^Ac	59.65 ± 2.12^Bab	57.08 ± 3.33^Aab	61.31 ± 1.97^ABbc	54.82 ± 2.72^Aa
2	75.54 ± 1.83^Ab	70.67 ± 2.01^Aa	77.21 ± 2.15^Ab	85.80 ± 2.94^Ac	95.98 ± 1.19^ABd	64.50 ± 4.23^Aab	70.77 ± 3.07^Ab	69.70 ± 2.96^Bab	64.76 ± 3.16^BCab	63.73 ± 3.57^Ba
6	77.29 ± 1.92^Ab	73.58 ± 1.15^ABa	80.24 ± 2.41^ABb	92.44 ± 1.68^Bc	94.42 ± 1.35^Ac	67.67 ± 2.48^Aa	71.62 ± 2.31^Aa	57.93 ± 2.77^Ab	55.00 ± 1.96^Abc	50.43 ± 2.32^Ac
12	79.93 ± 1.55^Ab	76.63 ± 1.08^Bab	75.56 ± 2.03^Aa	87.33 ± 2.26^ABc	97.35 ± 0.38^Bd	64.09 ± 2.48^Aa	72.01 ± 4.53^Aa	72.01 ± 4.53^Ba	66.84 ± 2.83^BCa	70.07 ± 3.60^Ba

Chewiness

Fresh	1.76 ± 0.23^ABa	2.13 ± 0.23^Bab	2.60 ± 0.35^Cb	2.57 ± 0.29^Cb	3.24 ± 0.36^Dc	2.80 ± 0.38^Aa	2.47 ± 0.34^Aa	2.48 ± 0.45^Aa	2.44 ± 0.26^Aa	2.16 ± 0.36^Aa
0	1.49 ± 0.16^Aa	1.63 ± 0.18^Aab	1.71 ± 0.16^Aab	1.99 ± 0.21^ABb	2.50 ± 0.26^BCc	4.09 ± 0.35^ABa	2.89 ± 0.45^ABb	2.47 ± 0.58^Abc	2.11 ± 0.32^Abc	1.89 ± 0.40^Ac
2	1.74 ± 0.12^ABab	1.63 ± 0.14^Aa	1.60 ± 0.22^Aa	1.67 ± 0.23^Aab	1.98 ± 0.12^Ab	4.65 ± 0.68^Ba	4.09 ± 0.80^BCab	3.36 ± 0.53^Abc	2.69 ± 0.47^Acd	2.12 ± 0.38^Ad
6	2.03 ± 0.17^BCa	1.93 ± 0.15^ABa	2.05 ± 0.17^ABa	2.23 ± 0.22^BCa	2.18 ± 0.15^ABa	4.35 ± 0.63^Ba	4.60 ± 0.51^Ca	2.42 ± 0.38^Ab	1.91 ± 0.20^Ab	1.73 ± 0.22^Ab
12	2.18 ± 0.11^Ca	2.27 ± 0.20^Bab	2.29 ± 0.19^BCab	2.68 ± 0.13^Cbc	2.81 ± 0.32^CDc	4.04 ± 0.67^ABa	4.28 ± 1.34^BCa	2.59 ± 0.60^Aab	2.78 ± 0.72^Aab	2.19 ± 0.49^Ab

Mean ± 95% CI. For each frozen storage time, different lowercase letters indicate significant differences ($p \leq 0.05$) as a result of the HPP pretreatment. For each HPP pretreatment, different capital letters indicate significant differences ($p \leq 0.05$) as a result of frozen storage time.

In agreement with Pita-Calvo et al. (2018b), who studied the effect of HPP pretreatment on the texture of raw and cooked hake after long-term storage, hardness values were higher in cooked albacore than in raw albacore for any storage time or HPP pretreatment. It could be related to the major water loss due to the cooking process (Mohan et al., 2015). HPP pretreatments at 200 MPa for 2–6 min led to a decrease in hardness of cooked albacore in comparison with the controls for any frozen storage. Torres et al. (2014) found similar results in horse mackerel. All frozen cooked controls showed higher hardness values than fresh samples ($p \leq 0.05$) for any frozen storage time. The same effect was observed in hake (Pita-Calvo et al., 2018b). However, samples pretreated at 200 MPa for 2–4 min presented non-noticeable changes in hardness with respect to fresh samples ($p > 0.05$), independently of the frozen storage. Therefore, these HPP pretreatments would allow obtaining fresh-like hardness values after cooking in raw albacore stored up to 12 months.

For any storage time, adhesiveness of raw albacore increased with HPP pretreatment (Table 6). Other authors also found higher adhesiveness values in other HPP-pretreated fish species than in the controls (Aubourg, Torres, et al., 2013; Torres et al., 2014). This adhesiveness increase was attributed to the unfolding of actin and sarcoplasmic proteins and the formation of hydrogen-bonded networks induced by pressure (Angsupanich & Ledward, 1998).

Cooking process strongly reduced the adhesiveness of albacore with respect to raw samples. For instance, after the cooking process, HPP-pretreated albacore at 200 MPa for 4 min at time 0 of frozen storage showed a 78.7% lower adhesiveness than before the cooking process. Similar results were observed in HPP-pretreated hake (Pita-Calvo et al., 2018b). Although in cooked albacore adhesiveness increased with HPP pretreatment, the highest obtained values remained low comparing with raw albacore. Thus, these variations in adhesiveness values would not result in appreciable changes in sensory texture. In this regard, other authors which found marked differences in adhesiveness of HPP-pretreated cooked horse mackerel, did not report negative changes in sensory texture at similar HPP conditions (150–300 MPa for 0–5 min) (Torres et al., 2014).

Springiness of raw HPP-pretreated albacore ranged between 71 and 97% (Table 6), similarly to the results found in fresh skipjack tuna (Jiranuntakul et al., 2018). There was not a clear effect of frozen storage on this texture parameter. Similarly, the effect of frozen

storage on other fish species, such as Atlantic mackerel and horse mackerel, was not evident (Aubourg, Torres, et al., 2013; Torres et al., 2014). Springiness of raw samples progressively increased with HPP pretreatment for any frozen storage time, likely due to the formation of hydrogen-bonded networks induced by pressure. Similar results were reported in HPP-pretreated hake (Pita-Calvo et al., 2018b). After cooking, similar springiness values were detected independently of frozen storage time or HPP pretreatment.

Chewiness of raw and cooked albacore (Table 6) presented a similar behavior to hardness, as previously reported Truong et al. (2016) in HPP-pretreated barramundi before freezing. In raw samples, chewiness gradually increased with HPP pretreatment in both fresh and thawed samples just after freezing (0 months). This effect was not evident from 2 months of frozen storage.

As with hardness, cooked albacore showed higher chewiness values than raw albacore. Pita-Calvo et al. (2018b) also reported a higher chewiness in cooked hake than in raw hake. In frozen samples, chewiness of cooked albacore decreased due to HPP pretreatment for any frozen storage time. Pretreated albacore steaks at 200 MPa for 2–6 min presented non-noticeable changes in chewiness with respect to fresh samples ($p > 0.05$) after 12 months of frozen storage. Therefore, these HPP pretreatments would allow obtaining fresh-like chewiness values in frozen albacore after thawing and cooking.

Based on these results, it could be concluded that the effect of HPP on texture of albacore strongly depends on whether it is raw or cooked. Texture changes after HPP pretreatment in raw material have been attributed to the formation of hydrogen-bonded networks (Angsupanich & Ledward, 1998; Chouhan et al., 2015), which would occur at pressures above 200 MPa (Alves de Oliveira et al., 2017). Whereas in cooked samples, HPP mediated changes could be attributed to the formation of a network dominated by hydrophobic and electrostatic bonds (Chouhan et al., 2015). This could explain the differences in texture parameter values observed between raw and cooked albacore.

Considering the overall impact on texture, HPP pretreatment at 200 MPa for 2–4 min would allow obtaining fresh-like springiness, hardness and chewiness in cooked albacore steaks frozen and stored during up to 12 months.

4.3.4. Salt-soluble protein content

Table 7 shows salt-soluble protein content of HPP-pretreated raw albacore before freezing and frozen storage, which indicates the extent of protein denaturation and aggregation (Cartagena et al., 2019).

Salt-soluble protein content decreased ($p \leq 0.05$) in all HPP-pretreated samples compared with the controls for any frozen storage time (0–12 months). Similar results were reported in HPP-pretreated sardine (Méndez et al., 2017) during up to 9 months of frozen storage. HPP-pretreated samples at 200 MPa for 6 min after 12 months of frozen storage showed the lowest salt-soluble protein content value (65.3% decrease in comparison with control samples at day 0 of frozen storage). Therefore, HPP pretreatment led to a marked protein denaturation and aggregation of salt-soluble proteins, as previously reported Tironi et al. (2007) in sea bass (*Dicentrarchus labrax* L.), which would explain the color and texture changes of albacore steaks caused by HPP pretreatment, both related to pressure-induced denaturation.

Table 7. Salt-soluble protein content (mg g^{-1}) of raw albacore steaks after HPP pretreatments (200 MPa for 0–6 min) before freezing and frozen storage time (up to 12 months). Fresh albacore steaks were also included for comparison.

	Control	Pressure/time (MPa/min)			
		200/0	200/2	200/4	200/6
Fresh	144.20 ± 8.16[Ac]	147.07 ± 8.20[Bc]	115.92 ± 8.57[Bab]	122.33 ± 12.13[Cb]	96.38 ± 11.58[Ca]
0	149.17 ± 13.30[Ac]	134.19 ± 8.81[ABc]	88.80 ± 2.99[Ab]	85.45 ± 5.45[Ab]	65.73 ± 4.22[ABa]
2	136.76 ± 7.92[Ac]	110.05 ± 7.00[Ab]	110.47 ± 9.33[Bb]	104.92 ± 8.30[BCb]	80.91 ± 9.49[BCa]
6	154.94 ± 14.62[Ad]	129.86 ± 16.53[ABc]	88.60 ± 5.17[Ab]	80.13 ± 6.99[Aab]	64.85 ± 6.90[ABa]
12	142.95 ± 16.03[Ad]	110.87 ± 13.86[Ac]	91.49 ± 7.73[Ac]	90.03 ± 5.17[ABb]	50.02 ± 3.72[Aa]

Mean values of three repetitions ± 95% CI. For each frozen storage time, different lowercase letters indicate significant differences ($p \leq 0.05$) as a result of the HPP pretreatment. For each HPP pretreatment, different capital letters indicate significant differences ($p \leq 0.05$) as a result of frozen storage time.

Cooked samples presented non-significant differences ($p > 0.05$) on salt-soluble protein content independently of the frozen storage time or the applied pretreatment conditions, reaching a value of 5.53 (0.33 CI 95) mg g^{-1} muscle. Therefore, the cooking process led

to an important decrease in salt-soluble protein in all samples with respect to results obtained in raw samples.

4.3.5. TBARS value

TBARS values of HPP-pretreated albacore before freezing and frozen storage are shown in Table 8. TBARS values ranged from 1.1 to 6.7 mg MDA kg^{-1} muscle, being similar to those obtained by Ben-Gigirey et al. (1999) and Cartagena et al. (2019). There was no effect of HPP pretreatment on TBARS value of albacore samples during the first 2 months of frozen storage. However, from 2 months of frozen storage, there was a decreasing trend in TBARS value ($p \leq 0.05$) with HPP pretreatment. At 200 MPa for 6 min, this parameter decreased after 6 and 12 months of frozen storage by 40.3 and 53.9%, respectively, with respect to the corresponding controls. These results are in agreement with Truong et al. (2016), who reported in barramundi a decrease in TBARS value after HPP pretreatment from 2 months of frozen storage. These authors suggested that enzyme activity related to lipid oxidation during frozen storage may be inhibited by HPP pretreatment (Truong et al., 2016). However, higher HPP treatments (400 MPa for 20 min) increased TBARS in cod (Angsupanich & Ledward, 1998). This increase has been attributed to the release of metallic ions which promote lipid oxidation, as a result of the pressure-induced denaturation of hemoproteins (Angsupanich & Ledward, 1998; Truong et al., 2016).

Table 8. TBARS values (mg MDA kg^{-1} muscle) of raw albacore steaks after HPP pretreatments (200 MPa for 0−6 min) before freezing and frozen storage time (up to 12 months). Fresh albacore steaks were also included for comparison.

		Pressure/time (MPa/min)			
	Control	200/0	200/2	200/4	200/6
Fresh	1.50 ± 0.39[Aa]	1.11 ± 0.26[Aa]	1.37 ± 0.27[Aa]	1.44 ± 0.34[Aa]	1.26 ± 0.20[Aa]
0	1.71 ± 0.30[Aab]	1.53 ± 0.34[Aab]	1.89 ± 0.33[Aa]	1.30 ± 0.12[Ab]	1.74 ± 0.30[Aab]
2	1.38 ± 0.14[Aa]	1.56 ± 0.30[Aab]	1.56 ± 0.15[Aab]	1.85 ± 0.22[Aab]	1.79 ± 0.22[Ab]
6	5.44 ± 0.66[Ba]	3.91 ± 0.66[Bb]	4.17 ± 0.47[Bb]	3.87 ± 0.57[Bb]	3.24 ± 0.26[Bb]
12	6.73 ± 0.06[Ba]	4.92 ± 1.00[Bab]	3.61 ± 0.02[Bb]	3.74 ± 0.17[Bb]	3.10 ± 0.57[Bb]

Mean values of three repetitions ± 95% CI. For each frozen storage time, different lowercase letters indicate significant differences ($p \leq 0.05$) as a result of the HPP pretreatment. For each HPP pretreatment, different capital letters indicate significant differences ($p \leq 0.05$) as a result of frozen storage time.

All albacore samples showed a marked increase ($p \leq 0.05$) in TBARS value between 2 and 6 months of frozen storage, independently of HPP pretreatment. In HPP-pretreated sardine at 200 MPa for 0 min, an increase in TBARS value was also observed between 3 and 6 months of frozen storage (Méndez et al., 2017).

There were no significant changes ($p > 0.05$) in TBARS value of cooked albacore as a result of both frozen storage and HPP pretreatment. The mean value of cooked albacore was 4.95 (0.05 CI 95) mg kg^{-1} muscle. Thus, the cooking process increased the TBARS value, probably masking the impact of HPP pretreatment and frozen storage on cooked samples.

In conclusion, after longer frozen storage times ($\geq$ 6 months) inherent increase of TBARS value due to the frozen storage was minimized by HPP pretreatment at 200 MPa for 2–6 min.

From these results, it can be concluded that HPP pretreatment at 200 MPa for 6 min before freezing allowed to maintain a similar thawing loss than the fresh control after up to 12 months of frozen storage, while in non-pretreated albacore it increased by 55.0% during the 12 months of frozen storage. Furthermore, from 6 months of frozen storage, this pretreatment minimized the increase in TBARS value inherent to frozen storage (53.9% decrease with respect to the control after 12 months of frozen storage). However, at 200 MPa for 6 min noticeable changes in color and texture were detected in raw albacore for any frozen storage time in comparison with non-pretreated albacore. After 12 months of frozen storage, L^* value and adhesiveness of HPP-pretreated albacore at 200 MPa for 6 min increased by 28.4 and 255.7%, respectively, with respect to the fresh control, while ΔE was higher than 13 units. Upon cooking, there were no changes in color due to the HPP pretreatment. Furthermore, after 12 months of frozen storage, HPP pretreatment at 200 MPa for 4 min allowed the maintenance of similar hardness, chewiness and springiness values to the fresh control, reducing thawing losses. Thus, HPP pretreatment before freezing could be a suitable technology for improving the quality of frozen albacore, especially when it is intended for its subsequent consumption once cooked or canning process. Further research is necessary to evaluate the impact of different freezing conditions on the quality of albacore.

4.4. Study 4: Application of high-pressure processing after freezing (before frozen storage) or before thawing in frozen albacore tuna (*Thunnus alalunga*)

Tuna industry is one of the most relevant and dynamic subsectors of fish production. Tuna vessels may generally remain at sea between 15 and 45 days until they return to port (Chust et al., 2019). In the long fishing expeditions, caught tuna is immediately frozen and stored onboard. After landing, tuna is generally thawed and finally processed (e.g., canned tuna). Although freezing is a good technological tool, quality changes occur during freezing, frozen storage, and thawing. Thus, the worldwide tuna fleet is actively seeking new approaches that can improve the quality of freezing tuna and its related products.

High-pressure processing (HPP) can be applied during freezing (pressure-shift freezing) (Alizadeh et al., 2007a; James et al., 2015; Tironi et al., 2007; Truong et al., 2015), during frozen storage (hyperbaric storage at sub-zero temperatures) (Fidalgo et al., 2014; Puértolas & Lavilla, 2020), or during thawing (high-pressure thawing) (Puértolas & Lavilla, 2020; Schubring et al., 2003; Tironi et al., 2007). All these processes showed promising results but they have not been industrialized due to their high cost (Barba et al., 2015b; Fidalgo et al., 2014; Puértolas & Lavilla, 2020; Truong et al., 2015).

High-pressure processing (HPP) as a pretreatment before freezing has been proposed as an alternative to conventional freezing (Cartagena et al., 2020b; Fidalgo et al., 2015; Méndez et al., 2017; Pazos et al., 2014, 2015; Torres et al., 2014; Truong et al., 2016) in order to improve the quality of frozen fish in comparison with conventional freezing. This method can improve the quality of frozen fish with respect to conventional freezing. For instance, it resulted in the inhibition of the development of lipid damage (Vázquez et al., 2013) or the enhancement of the sensorial quality (Torres et al., 2014) in several fish species.

HPP could be applied not only during thawing (high-pressure thawing) but also before thawing. Similarly, HPP could also be applied in frozen fish before frozen storage instead of during storage (hyperbaric storage). Both approaches could be of the main interest for the worldwide tuna fleet and the related industry. In the first approach, tuna would be frozen onboard immediately after catch and then frozen stored until vessels return to port. Once landing, tuna would be HPP-pretreated and finally thawed and processed. In the second approach, tuna would be frozen onboard immediately after caught, HPP-pretreated and finally frozen stored. Once landing, tuna would be thawed and processed.

The aim of this work was to evaluate the application of HPP in frozen albacore tuna as a pretreatment before the frozen storage (just after freezing) or before thawing, in order to reduce thawing loss while minimizing the negative effects of these conventional processes on fish quality, and comparing them with other processes based on pressure (pressure-assisted thawing, hyperbaric storage, etc.).

4.4.1. Thawing loss

Figure 37 shows thawing loss of HPP-pretreated samples before frozen storage (BFS) and before thawing (BT). Thawing loss values of albacore steaks were similar to those previously obtained at similar frozen storage times (up to 2 months) (Cartagena et al., 2020b). Both HPP pretreatments (200/6 and 600/0) resulted in a lower thawing loss ($p \leq 0.05$) than the controls regardless of whether it was applied before frozen storage (BFS) or before thawing (BT). After 45 days of frozen storage, thawing loss of 600/0 BT samples was $1.0 \pm 0.1\%$ whereas in the controls it was $5.0 \pm 0.4\%$. Thus, 600/0 BT decreased thawing loss by 79.7% with respect to the control. A similar decreasing effect was previously observed on HPP-pretreated albacore before freezing (200 MPa for 6 min) after similar frozen storage times (up to 2 months) (Cartagena et al., 2020b). Histological micrographs of several seafood products after similar HPP treatment showed muscle compaction that is characterized by a decrease in the extracellular space (Briones-Labarca et al., 2012; Chéret et al., 2005), as well as protein gelation characterized by the presence of holes in muscle (Briones-Labarca et al., 2012; Xuan et al., 2018) due to the formation of new intra- and intermolecular bonds induced by pressure (Rastogi et al., 2007). Changes in muscle protein structure as a consequence of pressures above 200 MPa, such as denaturation, aggregation, or gelatinization, would lead to better water retention (Chéret et al., 2005; Puértolas & Lavilla, 2020; Rastogi et al., 2007). All of these phenomena would explain the reduction of thawing losses presented in the current study and in the bibliography. High-pressure thawing at 200 MPa for up to 60 min also decreased thawing loss compared with conventional thawing in redfish (*Sebastes marinus*), haddock (*Melanogrammus aeglefinus*), and whiting (*Merlangius merlangius*) (Schubring et al., 2003). *Longissimus dorsi* pork subjected to pressure shift freezing (200 MPa) also resulted in lower thawing losses than samples frozen by conventional freezing methods (Hansen et al., 2003). Alizadeh et al. (2007b) suggested that the thawing time reduction caused by high-pressure thawing would involve less cell damage than

conventional thawing, decreasing thawing loss. By contrast, other authors did not find differences in thawing loss between high-pressure thawing and conventional process in several fish species, such as whiting and Atlantic salmon (Chevalier et al., 1999; Zhu et al., 2004).

□ Control ☐ 200/6 BFS ▨ 200/6 BT ▨ 600/0 BFS ▨ 600/0 BT
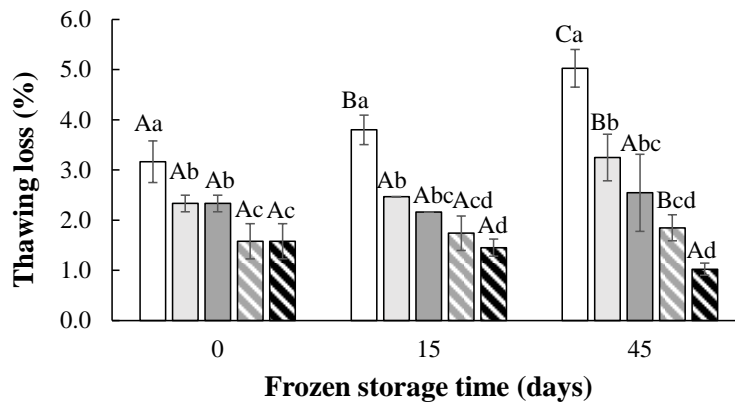
Figure 37. Thawing loss of HPP-treated frozen albacore at 200 MPa for 6 min (200/6) and 600 MPa for 0 min (600/0) before frozen storage (BFS) and before thawing (BT). For each frozen storage time, different lowercase letters indicate significant differences ($p \leq 0.05$) as a result of the HPP pretreatment. For each HPP pretreatment, different capital letters indicate significant differences ($p \leq 0.05$) as a result of frozen storage time.

Thawing loss of control albacore increased by 58.9% ($p \leq 0.05$) after 45 days of frozen storage. In 200/6 BFS and 600/0 BFS samples, the thawing loss after 45 days of frozen storage only increased by 39.3% and 17.1%, respectively. By contrast, in 200/6 BT and 600/0 BT samples, thawing loss remained stable ($p > 0.05$) throughout 45 days of frozen storage. These differences between the two processes could be explained by the fact that in BFS samples, the temperature increase during the HPP treatment (adiabatic heat) involve a temperature fluctuation during the frozen storage, leading to recrystallization, which would negatively affect thawing loss (Alves de Oliveira et al., 2017). Therefore, HPP pretreatment before thawing allowed bettering the minimization of thawing loss during the frozen storage than HPP before frozen storage.

4.4.2. Color

Figure 38 shows color parameters of HPP-pretreated albacore before frozen storage (BFS) and before thawing (BT). *L** and *b** values of the controls after 0 and 45 days of frozen storage were similar to those previously observed after 0 and 2 months of frozen storage (Cartagena et al., 2020b). However, at time 0 of frozen storage, HPP pretreatment

at 200/6 resulted in higher L^*, b^*, and ΔE values (60.3, 9.8, and 22 units, respectively) than those observed in HPP-pretreated albacore at 200 MPa for 6 min before freezing (52.7, 8.0, and 12.8 units, respectively) (Cartagena et al., 2020b). Thus, HPP affects more the color parameters when the pretreatment is applied in frozen albacore than once it is applied in fresh (non-frozen) products.

L^* value increased ($p \leq 0.05$) with the HPP pretreatment, regardless of whether HPP was applied before frozen storage or before thawing (Figure 38a). An increase in L^* value due to HPP treatment was also reported in fresh albacore, turbot, hilsa (*Tenualosa ilisha*), cod *Gadus morhua*), Atlantic mackerel (*Scomber scombrus*), and Atlantic salmon (Cartagena et al., 2019; Chouhan et al., 2015; Christensen et al., 2017). The same effect was found also on several high-pressure thawed fish and meat products (Alizadeh et al., 2007b; Schubring et al., 2003; Zhu et al., 2004). Pressure-induced denaturation of myofibrillar proteins causes an increase in L^* value as a result of HPP treatment, which has been reported to occur from around 200 MPa for 2 min (Alves de Oliveira et al., 2017; Chouhan et al., 2015; Christensen et al., 2017). In general, L^* value did not change ($p > 0.05$) throughout the frozen storage. Similar results were found in HPP-pretreated barramundi (*Lates calcarifer*) before freezing (Truong et al., 2016).

600/0 BT samples showed higher L^* values ($p \leq 0.05$) than those pretreated at 600/0 BFS. Thus, after 15 and 45 days of frozen storage, L^* value of 600/0 BT samples increased by 8.6 and 5.5%, respectively, with respect to 600/0 BFS ones.

600/0 BT decreased a^* value in comparison with the control (Figure 38b). Lower a^* values ($p \leq 0.05$) than in the controls were also found in 600/0 BFS samples at 45 days of frozen storage. In 200/6 BFS and 200/6 BT samples, a^* value did not change substantially with respect to control. Only significantly higher a^* values ($p \leq 0.05$) were found in 200/6 BT at 45 days of frozen storage. The effect of HPP on a^* value is reported to be highly dependent on processing conditions and fish species (Truong et al., 2015), which could explain these heterogeneous results.
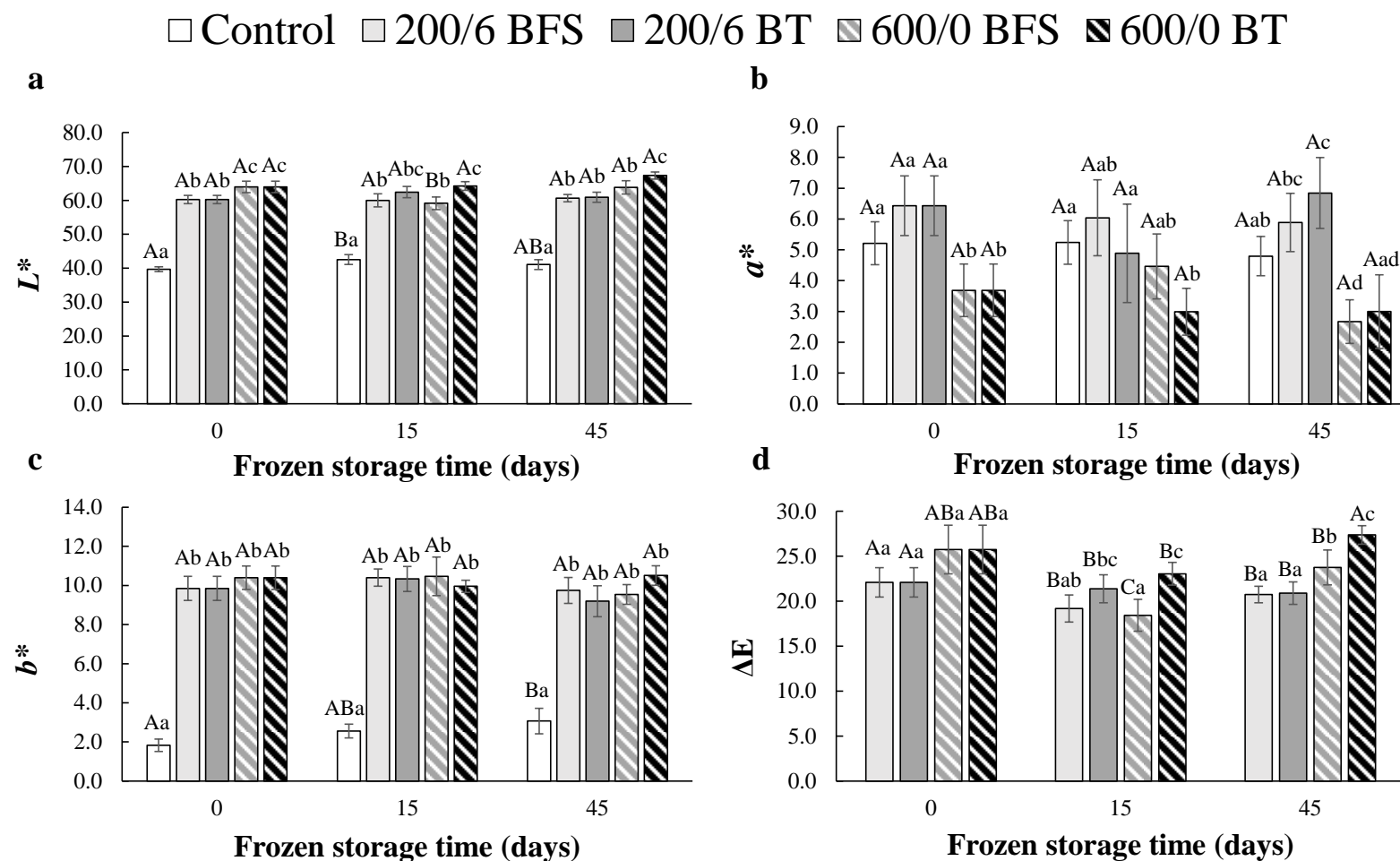
Figure 38. L^* (a), a^* (b) and b^* (c) values, and total color difference (ΔE) (d) of HPP-treated frozen albacore at 200 MPa for 6 min (200/6) and 600 MPa for 0 min (600/0) before frozen storage (BFS) and before thawing (BT). For each frozen storage time, different lowercase letters indicate significant differences ($p \leq 0.05$) as a result of the HPP pretreatment. For each HPP pretreatment, different capital letters indicate significant differences ($p \leq 0.05$) as a result of frozen storage time.

All HPP-pretreated albacore steaks showed a markedly higher ($p \leq 0.05$) $b*$ value than the controls (Figure 38c). Higher $b*$ values due to HPP treatment were also reported in several high-pressure thawed fish species (Schubring et al., 2003; Zhu et al., 2004). These changes in $b*$ value could be due to the denaturation of sarcoplasmic and myofibrillar proteins (Chouhan et al., 2015) or the lipid oxidation development (Torres et al., 2014).

Changes due to frozen storage were only evident in control samples, which showed a progressive increase in $b*$ value with the frozen storage (67.5% from 0 to 45 days). In HPP-pretreated samples, $b*$ value remained stable during the frozen storage.

ΔE values of all HPP-pretreated samples were higher than 10 units (Figure 38d). Thus, there were visually appreciable changes in color (Jung et al., 2003). Similar results were found in several high-pressure thawed fish species at 200 MPa (Schubring et al., 2003). At 200/6, there were no differences ($p > 0.05$) between applying HPP before frozen storage (BFS) or before thawing (BT). However, 600/0 BT resulted in higher ΔE values ($p \leq 0.05$) than 600/0 BFS.

In conclusion, the application of 200/6 and 600/0 HPP pretreatments in frozen albacore before frozen storage or before thawing resulted in noticeable changes in color parameters in all samples when compared with the controls. These changes were more marked than when similar HPP pretreatments were carried out in fresh albacore (before freezing) (Cartagena et al., 2020b).

4.4.3. Texture

Figure 39 shows texture parameters of HPP-pretreated frozen albacore before frozen storage and before thawing. All controls showed similar hardness, adhesiveness, springiness, and chewiness values to those previously observed in HPP-pretreated albacore before freezing (Cartagena et al., 2020b). However, hardness and chewiness of 200/6 BFS and 200/6 BT samples were much higher than when the same HPP pretreatment was applied before freezing (in fresh albacore) (Cartagena et al., 2020b)
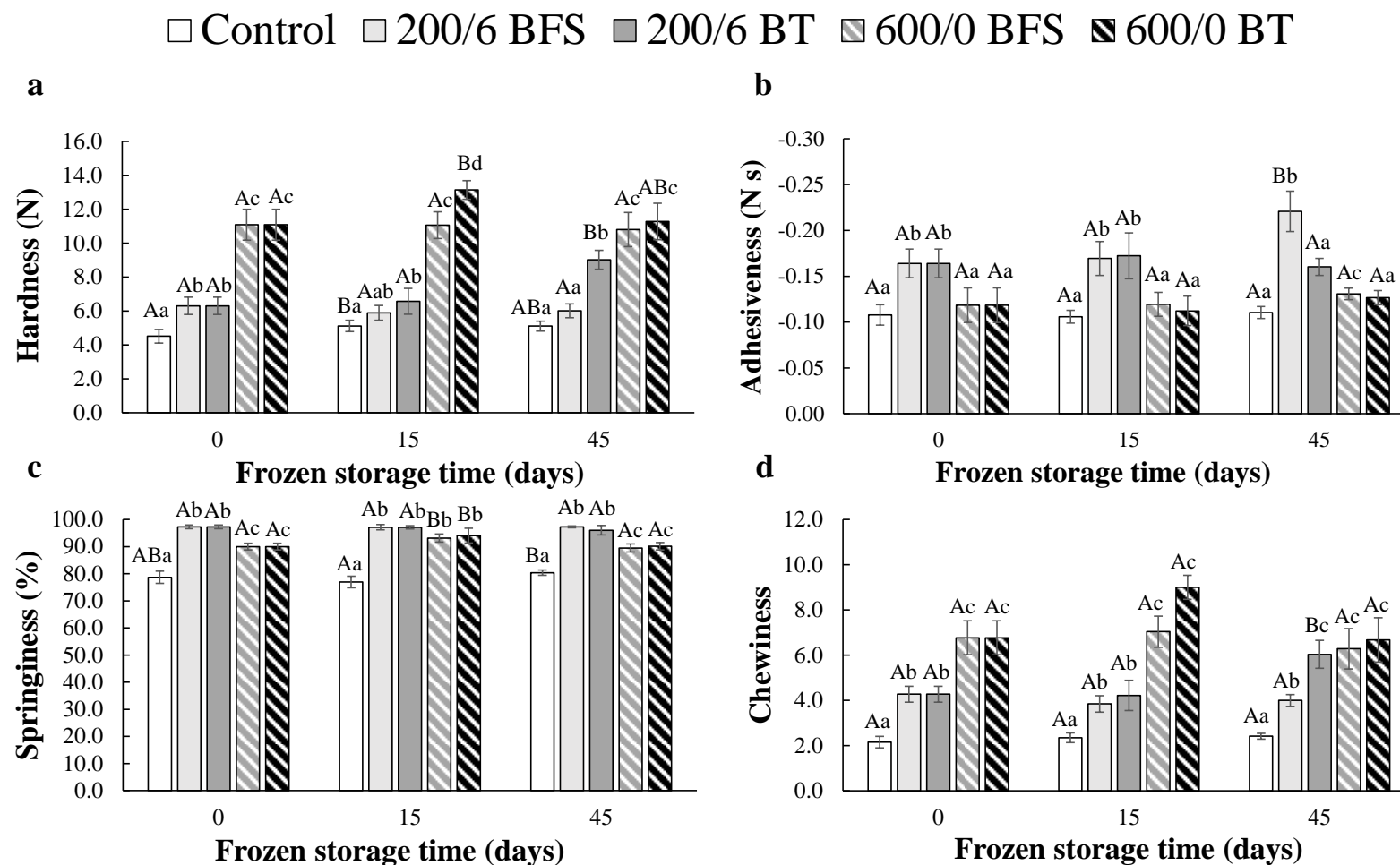
Figure 39. Hardness (a), adhesiveness (b), springiness (c), and chewiness (d) of HPP-treated frozen albacore at 200 MPa for 6 min (200/6) and 600 MPa for 0 min (600/0) before frozen storage (BFS) and before thawing (BT). For each frozen storage time, different lowercase letters indicate significant differences ($p \leq 0.05$) as a result of the HPP pretreatment. For each HPP pretreatment, different capital letters indicate significant differences ($p \leq 0.05$) as a result of frozen storage time.

All 600/0 pretreatments resulted in a marked increase in hardness ($p \leq 0.05$) with respect to the controls (Figure 39a). 200/6 BT led to a significant increase ($p \leq 0.05$) in hardness when compared with the control. For example, after 0 days of frozen storage, 200/6 BT resulted in a 39.8% higher hardness ($p \leq 0.05$) than the control. Higher hardness values than in the controls were also reported in several frozen fish species subjected to high-pressure thawing at similar pressures (200 MPa) (Alizadeh et al., 2007b; Schubring et al., 2003). By contrast, in 200/6 BFS samples there were no differences ($p > 0.05$) with the controls. Thus, 200/6 BFS allowed bettering the retention of the characteristic hardness of non-treated albacore than 200/6 BT. The increase in hardness caused by pressure was attributed to denaturation and aggregation of myofibrillar proteins (Chouhan et al., 2015). In agreement, other authors observed in differential scanning calorimetry thermograms and electrophoresis patterns that the peaks corresponding to myosin started to decreased between 200 and 300 MPa in several fish species (Angsupanich & Ledward, 1998; Christensen et al., 2017; Parniakov et al., 2018; Qiu et al., 2013, 2014; Tironi et al., 2007), whereas it was fully denatured around 400–500 MPa (Angsupanich & Ledward, 1998; Christensen et al., 2017). The compaction of fish muscle and the formation and protein gel networks could also contribute to this increase in hardness (Chéret et al., 2005; Puértolas & Lavilla, 2020). Truong et al. (2016) reported that hardness decreases during the frozen storage due to the endogenous proteolytic enzyme's activity. In HPP-pretreated samples before the frozen storage, the initial hardness increase caused by pressure would vanish during the frozen storage.

In HPP-pretreated albacore after 0 days of frozen storage, differences in adhesiveness were only found at 200/6, with higher values (52.3%) than the control (Figure 39b). Similarly, an increase in adhesiveness in comparison with the controls was observed in HPP-pretreated albacore before freezing under the same HPP conditions (Cartagena et al., 2020b), which was related to the unfolding of actin and sarcoplasmic proteins (Angsupanich & Ledward, 1998). All HPP-pretreated samples at 600/0 showed similar adhesiveness values ($p > 0.05$) to the controls, independently of the frozen storage time and whether HPP pretreatment was applied before frozen storage or before thawing. Differences between the application of HPP as a pretreatment before the frozen storage or before thawing were only found at 45 days of frozen storage, where 200/6 BFS resulted in a higher adhesiveness ($p \leq 0.05$) than 200/6 BT.

All HPP pretreatments, before frozen storage or before thawing, resulted in a higher springiness ($p \leq 0.05$) than the controls (Figure 39c). Similar results were found in several HPP-pretreated fish species before freezing (Cartagena et al., 2020b; Pita-Calvo et al., 2018a, 2018b; Torres et al., 2014). In general, this increase in springiness values was slightly higher ($p \leq 0.05$) at 200/6 (around 20% higher than the controls) than at 600/0 (around 15% higher than the controls). Finally, there was no impact ($p > 0.05$) on springiness of applying HPP as a pretreatment before the frozen storage or before thawing.

Chewiness changed in the same manner as hardness (Figure 39d), coinciding with findings reported in other fish species (Cartagena et al., 2019, 2020b; Chouhan et al., 2015; Pita-Calvo et al., 2018b). All HPP-pretreated samples showed higher chewiness values ($p \leq 0.05$) than the controls. Springiness and chewiness changes induced by pressure may be due to the unfolding of actin and sarcoplasmic proteins and formation of hydrogen-bonded networks (Angsupanich & Ledward, 1998; Chouhan et al., 2015).

In conclusion, all HPP pretreatments resulted in noticeable changes in all texture parameters with respect to the controls. The characteristic texture of non-treated albacore was much more affected by HPP pretreatment at 200/6 when it was applied in frozen samples than when it was applied in fresh ones before freezing (Cartagena et al., 2020b).

4.4.4. Salt-soluble protein content

Figure 40 shows salt-soluble protein content of HPP-pretreated frozen albacore before frozen storage and before thawing. All HPP-pretreated samples showed a lower salt-soluble protein content than the controls ($p \leq 0.05$), showing denaturation and aggregation of salt-soluble protein content, where myofibrillar proteins are the predominant ones (Cartagena et al., 2019). Thus, the color and texture changes observed in albacore samples could be related to the denaturation of muscle proteins induced by pressure, as previously described by Zhu et al. (2008). These changes in proteins are related to the high sensitivity of electrostatic and hydrophobic interactions to pressure, which maintain the quaternary, tertiary, and secondary structures of proteins (Alves de Oliveira et al., 2017; Fidalgo et al., 2015). A decrease in salt-soluble protein content due to the pressure was also reported in albacore and sardine (*Sardina pilchardus*) HPP-pretreated before freezing at 200 MPa for 6 min and 0 min, respectively (Cartagena et al., 2020b; Méndez et al., 2017), high-pressure thawed seabass at 200 MPa (Tironi et al., 2007), and turbot subjected to pressure

shift freezing at 140 MPa (Chevalier et al., 2000b). All HPP samples showed a lower salt-protein content than the controls after 15 and 45 days of frozen storage. This fact suggests a greater protein aggregation of myofibrillar proteins of HPP-pretreated samples throughout the frozen storage in comparison with the controls. The same effect was observed in HPP-pretreated sardine before freezing after up to 9 months of frozen storage (Méndez et al., 2017). Protein gelation as a consequence of protein denaturation and aggregation would allow bettering the retention of water (Chéret et al., 2005; Xuan et al., 2018). This phenomenon could explain the decreasing effect of HPP pretreatment on thawing losses after the frozen storage.
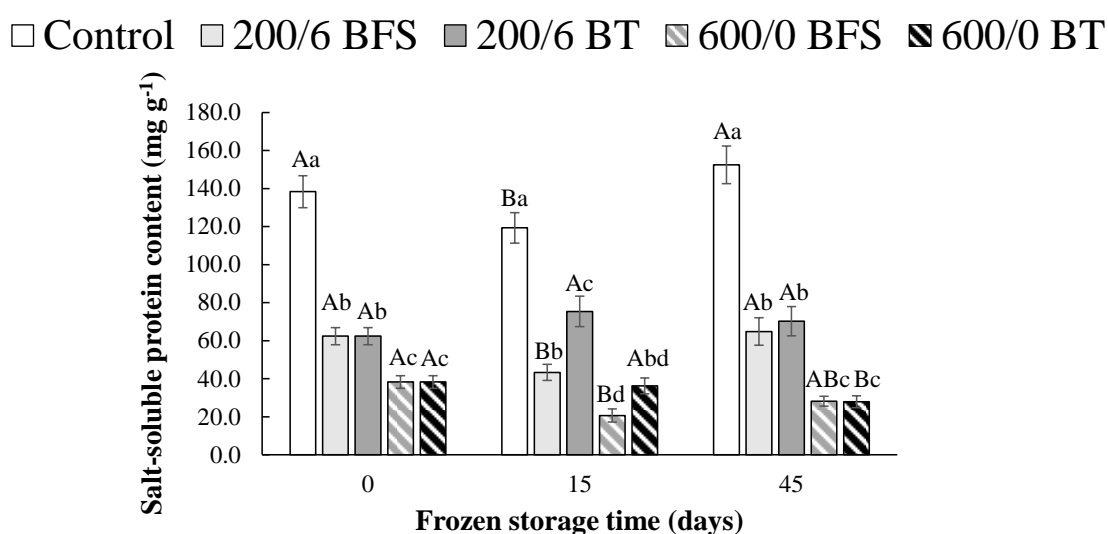
Figure 40. Salt-soluble protein content of HPP-treated frozen albacore at 200 MPa for 6 min (200/6) and 600 MPa for 0 min (600/0) before frozen storage (BFS) and before thawing (BT). For each frozen storage time, different lowercase letters indicate significant differences ($p \leq 0.05$) as a result of the HPP pretreatment. For each HPP pretreatment, different capital letters indicate significant differences ($p \leq 0.05$) as a result of frozen storage time.

Comparing BFS pretreatments with BT ones, differences were only found at 200/6 for 15 days of frozen storage, where 200/6 BFS showed a significantly lower salt-soluble protein content ($p \leq 0.05$) than 200/6 BT.

4.4.5. TBARS value

Figure 41 shows TBARS values of HPP-pretreated frozen albacore before frozen storage (BFS) and before thawing (BT). At time 0 of frozen storage, there was no impact of HPP pretreatment ($p > 0.05$) on TBARS value. Controls and 200/6 BFS samples thawed after up to 45 days of frozen storage showed similar TBARS values to those observed in HPP-pretreated albacore before freezing (Cartagena et al., 2020b). However, 200/6 BT samples

after 45 days of frozen storage showed markedly higher TBARS values than HPP-pretreated albacore before freezing after 2 months of frozen storage (Cartagena et al., 2020b). Lipid oxidation depends on several factors inherent to quality of raw material, such as age, chemical composition, fat profile, or handling (Alves de Oliveira et al., 2017; Puértolas & Lavilla, 2020). This fact could explain the high variability of these results.
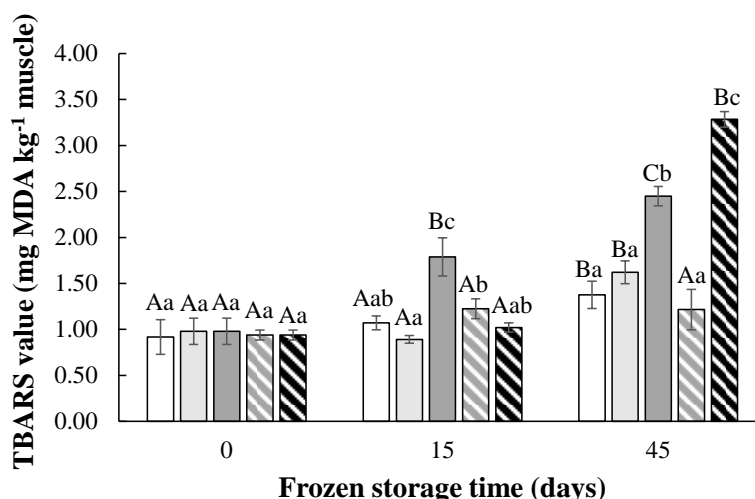
Figure 41. TBARS values of HPP-treated frozen albacore at 200 MPa for 6 min (200/6) and 600 MPa for 0 min (600/0) before frozen storage (BFS) and before thawing (BT). For each frozen storage time, different lowercase letters indicate significant differences ($p \leq 0.05$) as a result of the HPP pretreatment. For each HPP pretreatment, different capital letters indicate significant differences ($p \leq 0.05$) as a result of frozen storage time.

Higher TBARS values were observed after longer frozen storage times. The increased activity of several enzymes involved in lipid hydrolysis with the frozen storage (Fidalgo et al., 2015; Pazos et al., 2015), as well as an increasing content of free fatty acids (Fidalgo et al., 2015; Vázquez et al., 2018), could result in the acceleration of lipid oxidation. However, HPP pretreatment could inhibit the activity of lipid hydrolysis enzymes (Vázquez et al., 2018), and thus decrease the lipid oxidation development during the frozen storage (Puértolas & Lavilla, 2020).

In HPP-pretreated albacore before the frozen storage, only 600/0 BFS samples thawed after 15 days of frozen storage showed slightly higher TBARS values ($p \leq 0.05$) than the non-treated albacore. Similarly, no differences were found between pressured-shift freezing turbot samples at 140 MPa and those conventionally frozen during up to 75 days of frozen storage (Chevalier et al., 2000b).

In HPP-pretreated albacore before thawing, higher TBARS values than in the controls were found in 200/6 BT (67.0% higher) and 600/0 BT samples after 15 (78.1% higher) and 45 days (138.8% higher) of frozen storage, respectively.

In general terms, HPP-pretreated samples before thawing showed higher TBARS values than those pretreated before the frozen storage. TBARS value of 200/6 BT samples increased by 100.8 and 51.1% after 15 and 45 days of frozen storage with respect to 200/6 BFS samples thawed after 15 and 45 days of frozen storage, respectively. In 600/0 samples at 45 days of frozen storage, TBARS value was 170.1% higher ($p \leq 0.05$) in 600/0 BT than in 600/0 BFS ones. HPP would allow inhibiting endogenous enzyme activity responsible for lipid hydrolysis during the frozen storage (Truong et al., 2015), and subsequently lipid oxidation of HPP-pretreated albacore steaks BFS would be inhibited in a greater extent than in HPP-pretreated samples BT. Thus, HPP pretreatment before frozen storage would minimize lipid oxidation development during frozen storage in comparison with HPP before thawing.

The application of HPP before thawing or before frozen storage reduced the thawing losses with respect to controls. Moreover, after 45 days of storage, HPP BT albacore samples presented lower thawing losses than the HPP BFS ones. For instance, after 45 days of frozen storage, thawing loss of 600/0 BT and 600/0 BFS samples were $1.0 \pm 0.1\%$ and $1.8 \pm 0.2\%$, whereas in the controls it was $5.0 \pm 0.4\%$. It is also remarkable that the application of HPP before thawing allowed keeping thawing loss stable during all frozen storage, while it increased up to 58.9% in the controls. Non-relevant differences were detected in the rest of the studied parameters (color, texture, salt-soluble protein content, and lipid oxidation) between the application of HPP before frozen storage or before thawing. Therefore, the use of HPP before thawing would allow obtaining better thawed albacore quality than the application of HPP before frozen storage.

Regarding the tested HPP pretreatments, although 600/0 decreased thawing loss to a major extent than 200/6, this pretreatment led to sharp changes in color than 200/6 (higher *L** and *b** values) and texture (higher hardness and chewiness). Therefore, according to the results, the application of 200/6 BT would be a compromise option for reducing thawing loss in albacore with minimal changes on the quality.

4.5. Study 5: Impact of different air blast freezing conditions on the physicochemical quality of albacore (*Thunnus alalunga*) pretreated by high-pressure processing

This work was focused on evaluating the effect of different air blast freezing conditions on quality of HPP-pretreated albacore steaks before freezing.

It is well known that the undesirable changes caused by the freezing process on fish quality are highly dependent on the freezing conditions. However, in most of the bibliography related to the application of HPP pretreatment before freezing, fish samples were frozen under one specific freezing temperature (Aubourg, Torres, et al., 2013; Cartagena et al., 2020b, 2021; Méndez et al., 2017; Pita-Calvo et al., 2018b; Truong et al., 2016). Recently, Carrera et al. (2020) studied the influence of the storage temperature (–10, –20 and –30 ℃) on the quality of HPP-pretreated hake (150 MPa) and frozen at –40 ℃, observing that lowering the storage temperature was more effective than the application of HPP. However, the possible influence of the freezing conditions on the quality of HPP-pretreated fish is still unexplored.

Hence, the aim of this work was to study the effect of different air blast freezing conditions on the quality of HPP-pretreated albacore before freezing.

4.5.1. Freezing curve analysis

Figure 42 shows representative temperature curves of the 4 freezing conditions assessed. The duration of the precooling, phase transition stage and subcooling stages are shown in Table 9. The mean value of the initial freezing point for all freezing conditions was –1.1 ± 0.1 ℃. Since most of the ice crystals are formed during the phase transition stage, its duration determines the size, shape and location of ice crystals (Qian et al., 2018; M. Zhang et al., 2018; Y. Zhang & Ertbjerg, 2019). In the present work, 20/1 showed the longest phase transition, being around 6 times longer than 50/5 (Table 9). This parameter indicates approximately the time in which 80% of water is frozen (H. W. Kim et al., 2017). Similar trends were also observed in precooling and subcooling stages.

Impact of different air blast freezing conditions on the physicochemical quality of albacore (*Thunnus alalunga*) pretreated by high-pressure processing
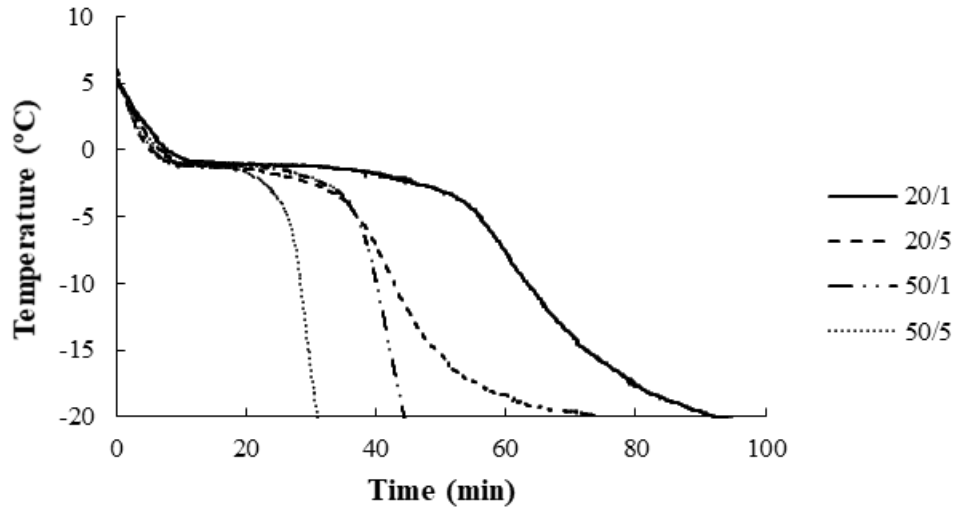
Figure 42. Representative freezing curves of albacore steaks frozen under different freezing conditions. 20/1 (−20 °C, 1 m s⁻¹); 20/5 (−20 °C, 5 m s⁻¹); 50/1 (−50 °C, 1 m s⁻¹); 50/5 (−50 °C, 5 m s⁻¹).

Characteristic freezing time, total freezing time, and freezing rate are also shown in Table 9 (Cartagena et al., 2020b; H. W. Kim et al., 2017; Y. H. B. Kim et al., 2015). As the phase transition stage, the characteristic freezing time was also longer in 20/1 than in the remaining freezing conditions. Regarding the freezing rate, the selected freezing conditions (air temperature and air velocity) allowed the obtaining of one low freezing rate (0.063 °C min⁻¹; 20/1), one high freezing rate (0.520 °C min⁻¹; 50/5) and two mid ones (0.191 °C min⁻¹, 20/5; 0.237 °C min⁻¹, 50/1) (Y. H. B. Kim et al., 2015).

Table 9. Characteristic parameters of freezing curves of albacore steaks frozen under differing freezing conditions. 20/1 (−20 °C; 1 m s⁻¹, 20/5 (−20 °C, 5 m s⁻¹), 50/1 (−50 °C, 1 m s⁻¹) and 50/5 (−50 °C, 5 m s⁻¹).

Freezing curve parameters	Freezing conditions			
	20/1	20/5	50/1	50/5
Precooling stage (min)	20.1 ± 8.0[a]	4.3 ± 0.2[b]	5.8 ± 3.1[b]	3.7 ± 1.5[b]
Phase transition stage (min)	72.8 ± 23.4[a]	27.8 ± 5.1[b]	21.5 ± 9.2[b]	12.9 ± 5.6[b]
Subcooling stage (min)	90.7 ± 37.3[a]	26.1 ± 0.4[b]	11.9 ± 7.3[b]	8.4 ± 4.1[b]
Characteristic freezing time (min)	94.7 ± 7.9[a]	31.6 ± 3.6[b]	25.7 ± 6.0[b]	10.9 ± 3.5[c]
Total freezing time (min)	183.6 ± 5.9[a]	58.2 ± 5.7[b]	39.9 ± 3.1[c]	24.9 ± 4.2[d]
Freezing rate (°C min⁻¹)	0.063 ± 0.015[a]	0.191 ± 0.022[b]	0.237 ± 0.055[b]	0.520 ± 0.183[c]

All values are means ± 95 CI (n = 2). Different capital letters in the same row indicate significant differences ($p \leq 0.05$).

4.5.2. Thawing, cooking and total weight losses

Figure 43 shows thawing loss, cooking loss and total weight loss of HPP and control albacore samples frozen under different freezing conditions (20/1, 20/5, 50/1, 50/5), after 2 and 9 months of frozen storage. The average thawing loss in the 50/5 samples (highest freezing rate, 0.520 ºC min^{-1}) was always lower than in the 20/1 ones (lowest freezing rate, 0.063 ºC min^{-1}). However, non-statistical differences ($p > 0.05$) were detected as a result of the freezing conditions for both, the HPP and the control samples (2 and 9 months of frozen storage). Other authors have described that fast freezing rates can reduce the thawing loss in seafood products such as crab sticks or Coho salmon *(Oncorhynchus kisutch)* and protein models (Kono et al., 2017; Otero et al., 2017; Y. Zhang & Ertbjerg, 2019). These different behaviors could be due to the use of more extreme minimum and maximum freezing rates than those studied in the present work (0.063 and 0.520 ºC min^{-1}, respectively). For instance, Kono et al. (2017) described differences in thawing loss in Coho salmon after applying freezing rates between 0.757 ºC min^{-1} (–35 ºC and 15 m s^{-1}), and 0.026 ºC min^{-1} (–25 ºC and 0 m s^{-1}).

It has been previously demonstrated that HPP pretreatment improves the quality of different fish products as long as HPP conditions (pressure and holding time) are properly selected (Aubourg, Torres, et al., 2013; Barba et al., 2015b; Cartagena et al., 2020b, 2020c; Grossi et al., 2016; Truong et al., 2016). As expected, HPP samples presented significantly lower thawing losses than the controls ($p \leq 0.05$), for all freezing conditions and frozen storage periods. For instance, thawing loss of HPP/9months samples before freezing at –50 ºC and 5 m s^{-1} after 9 months of frozen storage was 40% lower than in the controls. This decrease has been attributed to several protein changes induced by HPP pretreatment, such as aggregation, gelation or denaturation, which starts to occur at around 200 MPa and results in better water retention (Chéret et al., 2005; Puértolas & Lavilla, 2020). The reduction of thawing loss by HPP is of the main interest for both, the seafood industry and consumers, since drip loss causes not only a reduction in weight of products but also a loss of soluble proteins (Cai et al., 2014).

In general terms, there were no differences ($p > 0.05$) in cooking loss among the samples, independently of the freezing conditions (20/1, 20/5, 50/1, 50/5) (Figure 43b). Higher differences between the lowest and the highest freezing rates could be necessary to minimize cooking loss (Q. Sun et al., 2019; M. Zhang et al., 2018). Cartagena et al. (2020b) also found no effect of HPP pretreatment at 200 MPa for 6 min on cooking loss

of albacore steaks. McArdle et al. (2010) observed no effect of HPP treatment below 300 MPa for 20 min on fresh beef, whereas higher pressures (400 MPa for 20 min), led to an increase in cooking loss.

	20/1	20/5	50/1	50/5
Control/2months	Aa	Aa	Aa	Aa
HPP/2months	Abc	Ab	Abc	Abc
Control/9months	Aab	Aab	Aab	Aab
HPP/9months	Ac	Ac	Ac	Ac

	20/1	20/5	50/1	50/5
Control/2months	Aa	Aa	Aab	Aa
HPP/2months	ABa	ABa	Aa	Ba
Control/9months	Aa	Aa	Ab	Aa
HPP/9months	Aa	Aa	Aab	Aa

	20/1	20/5	50/1	50/5
Control/2months	Aa	Aa	Aa	Aa
HPP/2months	ABab	Ab	Ba	Aa
Control/9months	Aab	Aab	Aa	Aa
HPP/9months	Ab	Ab	Aa	Aa
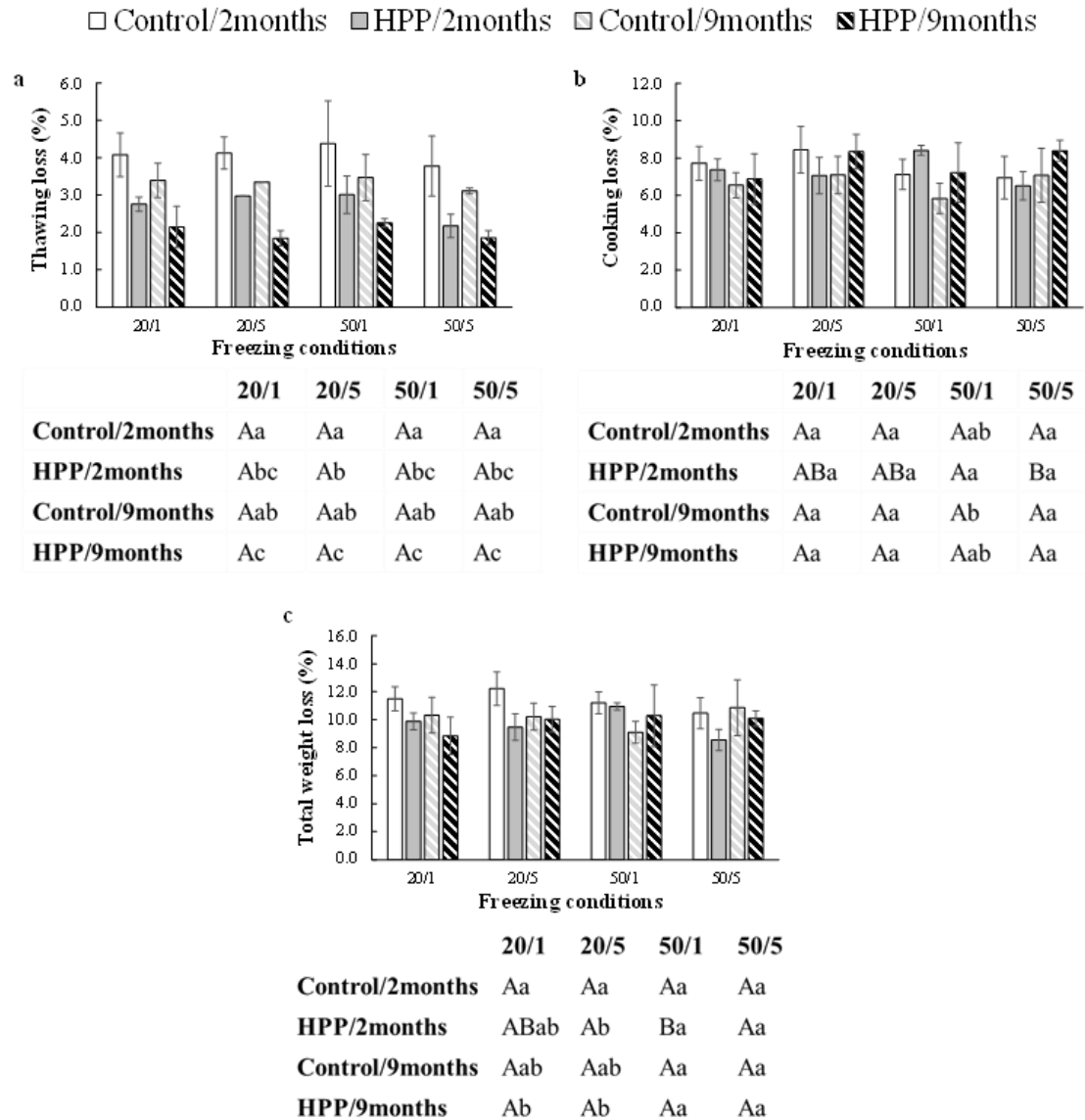
Figure 43. Thawing loss (a), cooking loss (b) and total weight loss (c) of HPP-pretreated (HPP; 200 MPa, 6 min) and control albacore steaks frozen under differing conditions, after 2 and 9 months of frozen storage. Freezing conditions: 20/1 (−20 °C; 1 m s^{-1}), 20/5 (−20 °C, 5 m s^{-1}), 50/1 (−50 °C, 1 m s^{-1}) and 50/5 (−50 °C, 5 m s^{-1}). Mean values of three replicates; errors bars indicate 95% CI. Different capital letters in the same raw indicate significant differences (p ≤ 0.05). Different lowercase letters in the same column indicate significant differences (p ≤ 0.05).

No significant differences (*p* > 0.05) were detected in total weight loss related to the freezing conditions, except for HPP/2months (Figure 43c). After 2 months of frozen storage, HPP samples had lower total weight losses than their respective controls for the

4 freezing conditions studied. HPP pretreatment at 200 MPa for 6 min before freezing at 50/5 (the fastest freezing rate tested) resulted in the lowest total weight loss value. By contrast, after 9 months there were no differences ($p > 0.05$) between all HPP pretreated samples and the controls. Thus, the positive effect of HPP pretreatment on total weight loss would disappear after long-term storage, as Cartagena et al. (2020b) previously reported. These authors observed no differences in total weight losses between HPP pretreated albacore at 200 MPa for 6 min and the controls after frozen storage periods longer than 2 months.

In conclusion, the freezing conditions analyzed (–20 ºC and 1 m s-1, –20 ºC and 5 m s-1, –50 ºC and 1 m s^{-1}, –50 ºC and 5 m s^{-1}) did not impact on the thawing and cooking losses, and the total weight loss. Independently of the freezing conditions, HPP allowed the reduction of thawing loss during up to 9 months of frozen storage. The lower average thawing loss and total weight loss were obtained in pretreated samples at 200 MPa for 6 min and then frozen at –50 ºC using an air velocity of 5 m s^{-1}. These processing conditions reduced both thawing and total weight losses by 40 and 18%, respectively, after 2 months of frozen storage.

4.5.3. Color

Figure 44 shows L^*, a^* and b^* values and total color difference (ΔE) of albacore (HPP, control) frozen at different conditions (20/1, 20/5, 50/1, 50/5) and thawed after 2 and 9 months of frozen storage. In general, there were no changes in L^* value due to the freezing conditions ($p > 0.05$). Similarly, Cai et al. (2014) did not find differences in L^* value between frozen sea bass (*Dicentrarchus labrax* L.) at –18 ºC and at –50 ºC. Kono et al. (2017) also reported similar L^* values after thawing in both fast and normal frozen Coho salmon. Some authors reported an increase in L^* value as a consequence of slow freezing methods (Q. Sun et al., 2019; M. Zhang et al., 2018). It could be probably related to the stronger muscle structure changes of slow freezing, which results in greater light dispersion (Q. Sun et al., 2019). HPP pretreatment resulted in higher L^* values ($p \leq 0.05$), regardless of the freezing conditions. It is well known that the application of HPP in fresh fish increases L^* value during refrigeration (Cartagena et al., 2019; Chouhan et al., 2015; Jiranuntakul et al., 2018; Puértolas & Lavilla, 2020; Ramirez-Suarez & Morrissey, 2006), and also after freezing, after frozen storage and after thawing (Cartagena et al., 2020b; Pita-Calvo et al., 2018b; Torres et al., 2014). This increase has been recently described in

frozen fish treated by HPP (Cartagena et al., 2020a). This increment in *L** has been attributed to the denaturation of myofibrillar proteins after HPP treatments above 200 MPa (Bak et al., 2019; Puértolas & Lavilla, 2020; Truong et al., 2015).

□ Control/2months ▨ HPP/2months ▨ Control/9months ◪ HPP/9months

	20/1	20/5	50/1	50/5
Control/2months	Aa	Aa	Aa	Aa
HPP/2months	Ab	Ab	Ab	Ab
Control/9months	Ac	Ac	Ac	Aa
HPP/9months	Ad	Bb	ABb	Bb

	20/1	20/5	50/1	50/5
Control/2months	Aa	Aa	Aa	Aa
HPP/2months	Aa	Ab	Aa	Aa
Control/9months	Aa	Aac	Aa	Aa
HPP/9months	Aa	Bbc	ABa	ABa

	20/1	20/5	50/1	50/5
Control/2months	Aa	Aa	Aa	Aa
HPP/2months	Ab	Ab	Ab	Ab
Control/9months	Ab	Ab	Ac	Aa
HPP/9months	Ac	Ab	Ad	Ab

	20/1	20/5	50/1	50/5
Control/2months	-	-	-	-
HPP/2months	Aa	Aa	Aa	Aa
Control/9months	-	-	-	-
HPP/9months	Aa	Bb	Aa	ABa

Figure 44. L* value (a), a* value (b), b* value (c) and total color difference (ΔE; d) of HPP-pretreated (HPP; 200 MPa, 6 min) and control albacore steaks frozen under differing conditions, after 2 and 9 months of frozen storage. Freezing conditions: 20/1 (−20 ℃; 1 m s⁻¹), 20/5 (−20 ℃, 5 m s⁻¹), 50/1 (−50 ℃, 1 m s⁻¹) and 50/5 (−50 ℃, 5 m s⁻¹). Mean values of three replicates; errors bars indicate 95% CI. Different capital letters in the same raw indicate significant differences (p ≤ 0.05). Different lowercase letters in the same column indicate significant differences (p ≤ 0.05).

From a general point of view, *a** value slightly rose with the increase of the freezing rate, although in general no statistical differences were found (*p* > 0.05) (Figure 44b). Similar results were found in sea bass frozen at −18 °C and −55 °C, after 3 months of frozen storage at −18 ℃ (Cai et al., 2014). Excluding 20/5 samples, there were no differences in *a** value (*p* > 0.05) as a consequence of the combination of HPP pretreatment and frozen

storage time (Figure 44b). Thus, a clear effect of HPP on redness could not be concluded, probably due to the high initial variability of redness among albacore samples. In agreement, other researchers reported a negligible effect of HPP pretreatment on $a*$ value in other fish species (Aubourg, Torres, et al., 2013; Torres et al., 2014).

There were no changes in $b*$ value ($p > 0.05$) of albacore because of the freezing conditions and their corresponding freezing rates (Figure 44c). Other authors have described similar results in carp (*Cyprinus carpio*), Coho salmon or porcine longissimus muscles (Kono et al., 2017; Q. Sun et al., 2019; M. Zhang et al., 2018). $b*$ value of all HPP-pretreated samples (200/6) was always higher than in the controls ($p \leq 0.05$), independently of the freezing conditions. Jiranuntakul et al. (2018) reported an increase in $b*$ value of HPP-treated fresh skipjack tuna (*Katsuwonus pelamis*) under similar HPP conditions (150 MPa for 5 min). Similar findings were also reported in hilsa (*Tenualosa ilisha*) (Chouhan et al., 2015). These changes in $b*$ value could be attributed to the denaturation of sarcoplasmic and myofibrillar proteins (Cartagena et al., 2020b; Chouhan et al., 2015).

In general, there was no effect of the freezing rate on ΔE value of albacore steaks (Figure 44d). Similarly, Zhu et al. (2004) did not report changes in ΔE values of Atlantic salmon (*Salmo salar*) due to the freezing conditions. ΔE value was markedly higher in all HPP-treated samples than in their respective controls. This color change could influence the consumer purchase intention, although these differences would disappear after cooking (Cartagena et al., 2020b).

In conclusion, the studied freezing conditions (20/1, 20/5, 50/1, 50/5) had no impact on the color characteristics of the albacore steaks. Independently of the freezing rate, adverse changes in color due to HPP pretreatment occurred (increase in $L*$ and $b*$ values with respect to the controls). However, it is necessary to remark that these color changes are not evident after cooking (Cartagena et al., 2020b). Furthermore, although instrumental color measurements showed some differences between HPP samples and their controls, the values obtained in HPP samples were close to the color range of fresh albacore (Ramirez-Suarez & Morrissey, 2006).

4.5.4. Texture

Figure 45 shows hardness, adhesiveness, springiness, and chewiness of HPP and control samples frozen under different freezing conditions and thawed after 2 and 9 months of frozen storage. In both HPP and control samples, lower hardness values were obtained in those frozen at 20/1 (the slowest freezing rate, 0.063 ℃ min^{-1}), with respect to 20/5, 50/1, and 50/5 ones. It is known that freezing causes a decrease of hardness in albacore (Cartagena et al., 2020b), so freezing at –50 ℃ would allow the obtaining of albacore steaks with a hardness more akin to fresh samples than 20/1. Qian et al. (2018) also observed that high freezing rates better retained the texture of bighead carp *(Aristichthys nobilis)* fillets than low freezing rates. Cai et al. (2014) noticed that sea bass samples frozen at –18 ℃ presented lower hardness values than those frozen at –50 ℃, after 3 months of frozen storage. These differences were attributed to the cellular damage caused by the higher growth of ice crystals as a result of slow freezing (Cai et al., 2014). On the other hand, L. Shi et al. (2018) reported no differences in hardness values as a result of the freezing temperature after 1 week of frozen storage in red swamp crayfish (*Procambarus clarkii*). The different impact of similar freezing conditions on hardness could be explained by, among other factors, the different frozen storage times.

Regarding the influence of the HPP pretreatment on hardness, overall, there were no differences ($p > 0.05$) in comparison with the controls. Similarly, Cartagena et al. (2020b) did not observe differences in hardness between HPP-pretreated albacore at 200 MPa for 6 min and the controls after similar frozen storage times (2 and 12 months). Muscle compaction induced by pressure promotes the formation of new intra- and intermolecular bonds in muscle proteins, leading to protein gelation (Truong et al., 2015). This protein gelation involves better water retention and results in a higher hardness of fresh samples (Chéret et al., 2005; Truong et al., 2015). However, in frozen samples, ice crystal formation may disrupt muscle fibers, affecting the texture properties of frozen products (Cartagena et al., 2020b). Hence, HPP pretreatments would offset the negative effect of freezing and frozen storage on texture.

Excluding HPP/2months samples, adhesiveness did not change as a consequence of the freezing conditions ($p > 0.05$), independently of the combination of HPP pretreatment and frozen storage time (Figure 45b). By contrast, all HPP samples showed higher adhesiveness values ($p \leq 0.05$) than their controls regardless of the freezing conditions.

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These results were similar to those previously observed in other HPP-pretreated fish species before freezing (Aubourg, Torres, et al., 2013; Cartagena et al., 2020b; Torres et al., 2014; Truong et al., 2016). The rise in adhesiveness has been ascribed to the unfolding of actin and sarcoplasmic proteins induced by pressure, and the consequent development of hydrogen-bonded networks (Puértolas & Lavilla, 2020).

□ Control/2months ■ HPP/2months ▨ Control/9months ◩ HPP/9months

	20/1	20/5	50/1	50/5
Control/2months	Aa	Ba	ABab	ABa
HPP/2months	Ab	Aa	Ab	Ab
Control/9months	Aa	ABa	Bb	ABab
HPP/9months	Aa	ABb	ABa	Ba

	20/1	20/5	50/1	50/5
Control/2months	Aa	Aa	Aa	Aa
HPP/2months	ABb	Ab	Bb	ABb
Control/9months	Ac	Ac	Ac	Ac
HPP/9months	Ad	Ad	Aa	Ad

	20/1	20/5	50/1	50/5
Control/2months	ABa	Aa	ABa	Ba
HPP/2months	Ab	Ab	Ab	Ab
Control/9months	Aa	Aa	Aa	Ba
HPP/9months	Ab	Ab	Ab	Ab

	20/1	20/5	50/1	50/5
Control/2months	Aa	Aa	Aa	Aa
HPP/2months	Ab	Ab	Ab	Ab
Control/9months	Aa	Aa	Aa	Aac
HPP/9months	Aa	Aa	Aa	Ac

Figure 45. Hardness (a), adhesiveness (b), springiness (c) and chewiness (d) of HPP-pretreated (HPP; 200 MPa, 6 min) and control albacore steaks frozen under differing conditions, after 2 and 9 months of frozen storage. Freezing conditions: 20/1 (−20 ºC; 1 m s^{-1}), 20/5 (−20 ºC, 5 m s^{-1}), 50/1 (−50 ºC, 1 m s^{-1}) and 50/5 (−50 ºC, 5 m s^{-1}). Mean values of three replicates; errors bars indicate 95% CI. Different capital letters in the same raw indicate significant differences (p ≤ 0.05). Different lowercase letters in the same column indicate significant differences (p ≤ 0.05).

In general, there were no noticeable differences in springiness values due to the freezing conditions (Figure 45c). Shi et al. (2018) did not find remarkable changes in springiness

of red swamp crayfish subjected to different freezing temperatures (from –80 to –18 ℃). However, springiness increased due to HPP pretreatment. All HPP samples showed significant differences in comparison with their controls ($p \leq 0.05$), independently of the freezing conditions. For instance, in samples processed at 200 MPa for 6 min before freezing at 20/1, springiness increased by about 20% with respect to their respective controls. Other authors found similar results (Cartagena et al., 2020b; Pita-Calvo et al., 2018b). Truong et al. (2016) related this increase of springiness to the formation of hydrogen-bonded networks in proteins induced by the pressure.

There was no effect ($p > 0.05$) of the freezing conditions on chewiness (Figure 45d). HPP/2months samples showed higher significant chewiness values than their respective controls ($p \leq 0.05$) independently of the freezing conditions. However, these differences disappeared after 9 months of frozen storage. Similar behavior has been previously reported by other authors after short frozen storage times (Cartagena et al., 2020b).

In conclusion, freezing conducted at –50 ℃ (50/1 and 50/5) allowed the achievement of higher and closer-to-fresh hardness values than the freezing at the lowest freezing rate (20/1), although it was only significant after 9 months of frozen storage. It was not detected any effect of the freezing conditions on adhesiveness, springiness, and chewiness. The application of the HPP pretreatment (200 MPa for 6 min) generally had no impact on hardness and chewiness, although an increase in adhesiveness and springiness was found with respect to the controls, independently of the freezing conditions.

4.5.5. Salt-soluble protein content

Figure 46 shows the salt-soluble protein content of HPP-pretreated albacore before freezing at different freezing conditions and thawed after 2 and 9 months of frozen storage. Salt-soluble proteins mainly correspond to myofibrillar proteins (L. Shi et al., 2018), so it is a good indicator of the impact of different processes on the muscular proteins (Cartagena et al., 2019).

For all HPP and control samples (2 and 9 months of frozen storage), the salt-soluble protein content did not change ($p > 0.05$) as a consequence of freezing conditions. It indicates a similar grade of denaturalization in myofibrillar protein, independently of the freezing rate. This result is in accordance with the no impact of freezing conditions on

thawing loss detected in this work. Nevertheless, some authors have reported differences in salt-soluble protein content depending on the freezing rate (Qian et al., 2018). These authors suggested that larger ice crystals formed during slow freezing methods would lead to greater disruption of myofibrillar proteins than the small ice crystals formed during fast freezing methods, which result in a higher concentration of solutes in the unfrozen water and thus involve a higher myofibrillar protein denaturation (Qian et al., 2018; Y. Zhang & Ertbjerg, 2019). Therefore, higher differences in freezing rates than those studied in the present work (0.063–0.520 ℃ min^{-1}) may be necessary to impact on albacore salt-soluble protein content.

☐ Control/2months ▨ HPP/2months ☐ Control/9months ◩ HPP/9months
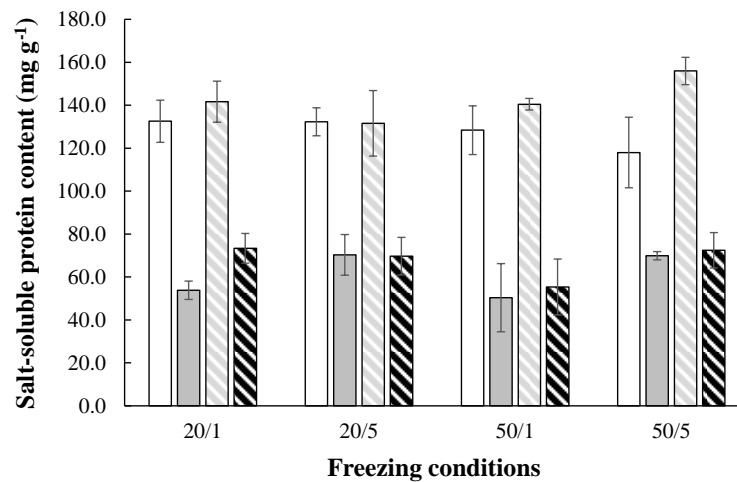
	20/1	20/5	50/1	50/5
Control/2months	Aa	Aa	Aa	Aa
HPP/2months	Ab	Ab	Ab	Ab
Control/9months	Aa	Aa	Aa	Ac
HPP/9months	Ac	Ab	Ab	Ab

Figure 46. Salt-soluble protein content of HPP-pretreated (HPP; 200 MPa, 6 min) and control albacore steaks frozen under differing conditions, after 2 and 9 months of frozen storage. Freezing conditions: 20/1 (–20 ℃; 1 m s^{-1}), 20/5 (–20 ℃, 5 m s^{-1}), 50/1 (–50 ℃, 1 m s^{-1}) and 50/5 (–50 ℃, 5 m s^{-1}). Mean values of three replicates; errors bars indicate 95% CI. Different capital letters in the same raw indicate significant differences (p ≤ 0.05). Different lowercase letters in the same column indicate significant differences (p ≤ 0.05).

All HPP pretreatments decreased salt-soluble protein ($p \leq 0.05$) in comparison with their controls, which indicates denaturation and aggregation of myofibrillar proteins (Cartagena et al., 2020b; Méndez et al., 2017). These changes in protein conformation induced by pressure may lead to protein gelation due to the formation of hydrogen-bonded

networks that involve several color and texture changes and an enhancement of water retention (Angsupanich & Ledward, 1998; Chéret et al., 2005). However, protein structural changes induced by heat treatment result in a different protein gel network, dominated by hydrophobic and electrostatic bonds (Angsupanich & Ledward, 1998).

4.5.6. TBARS value

Figure 47 shows TBARS value of HPP-pretreated albacore before freezing at different freezing conditions. TBARS values ranged from 1.95 to 4.89 mg kg^{-1}. These values could be considered high. Ben-Gigirey et al. (1999) described a TBARS value of 2.38 mg kg^{-1} in fresh albacore. This value is similar to those obtained in the present study after 2 months of frozen storage. However, the values obtained after 9 months of frozen storage were higher because lipid oxidation increased during the frozen storage.

In general, freezing conditions did not exert any effect on TBARS value. Only control/9 months samples showed some significant differences ($p \leq 0.05$). However, the values among the 4 freezing conditions were very similar, ranging from 4.32 ± 0.24 to 4.89 ± 0.07 mg kg^{-1}. Similarly, Soyer et al. (2010) did not find differences in TBARS value between chicken meat samples frozen at –7, –12 and –18 ºC. By contrast, H. W. Kim et al. (2017) reported higher TBARS values in frozen chicken meat samples subjected to slow freezing (conventional air freezer at –30 ºC) than in those subjected to fast freezing (liquid nitrogen chamber at –70 ºC). Greater cell membrane damage caused by larger ice crystals inherent to slow freezing would lead to the release of pro-oxidant substances (H. W. Kim et al., 2017), promoting lipid oxidation. Faster freezing rates than those assessed in the present work could lead to a decrease in TBARS value.

In general, no significant differences in TBARS value ($p > 0.05$) were found between HPP and control samples for any freezing condition. (Méndez et al., 2017) also found no clear effect of HPP pretreatment on TBARS value of frozen sardine (*Sardina pilchardus*). However, other authors found in HPP-treated fish a decrease (Cartagena et al., 2020b; Ramirez-Suarez & Morrissey, 2006; Truong et al., 2016) or an increase (Jiranuntakul et al., 2018) of TBARS value. These differing results at similar processing conditions and even in the same species could be caused by several factors such as the different conditions during catching or handling on boats, the age of the fish or the individual

differences in fat composition (Jiranuntakul et al., 2018; Puértolas & Lavilla, 2020; Truong et al., 2015).
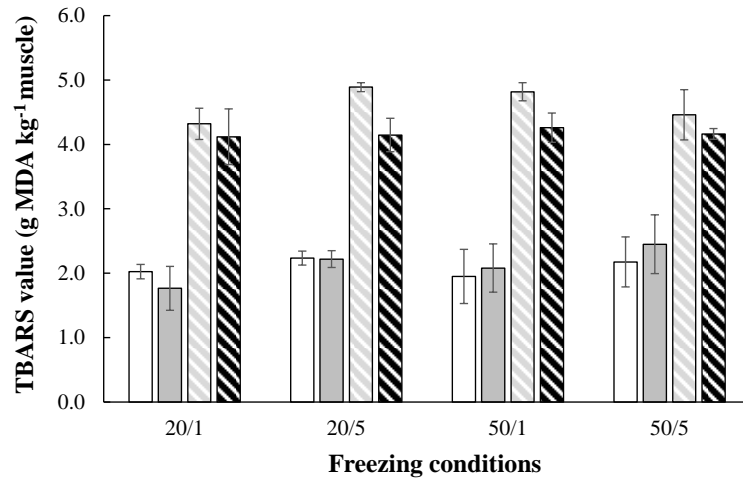
	20/1	20/5	50/1	50/5
Control/2months	Aa	Aa	Aa	Aa
HPP/2months	Aa	Aa	Aa	Aa
Control/9months	Ab	Bb	Bb	ABb
HPP/9months	Ab	Ac	Ab	Ab

Figure 47. TBARS value of HPP-pretreated (HPP; 200 MPa, 6 min) and control albacore steaks frozen under differing conditions, after 2 and 9 months of frozen storage. Freezing conditions: 20/1 (−20 ℃; 1 m s^{-1}), 20/5 (−20 ℃, 5 m s^{-1}), 50/1 (−50 ℃, 1 m s^{-1}) and 50/5 (−50 ℃, 5 m s^{-1}). Mean values ± 95 CI. Errors bars indicate 95% CI. Different capital letters in the same raw indicate significant differences (p ≤ 0.05). Different lowercase letters in the same column indicate significant differences (p ≤ 0.05).

In both HPP-pretreated and control samples, freezings conducted at −50 ℃ allowed the achievement of higher and closer-to-fresh hardness value than the freezing at −20 ℃ and 1 m s^{-1}. After 9 months of frozen storage, the HPP-pretreated samples before freezing at the slowest (−20 ℃ and 1 m s^{-1}) and the fastest (−50 ℃ and 5 m s^{-1}) freezing conditions presented important thawing loss reductions with respect to their controls (37 and 40%, respectively). Thus, HPP could be used for reducing the thawing loss of frozen albacore, independently of the air blast freezing conditions. Considering the conditions and parameters tested, HPP pretreatment (200 MPa, 6 min) followed by freezing at −50 ℃ (at 1 or 5 m s^{-1}) would allow to achieve the best results in albacore quality after up to 9 months of frozen storage.

The presented conclusions should be interpreted considering the limited number of individuals used in this work. Industrial scale studied are needed to confirm them. The

evaluation of the possible combination of HPP pretreatments with other freezing technologies characterized by higher freezing rates, such as cryogenic immersion or ultrasound assisted freezing, could be considered for further studies.

4. GENERAL DISCUSSION

This chapter will discuss the potential benefits of high-pressure processing (HPP) for improving the quality of albacore (*Thunnus alalunga*), and more specifically, for decreasing weight losses during processing and storage, which is the main purpose of the present work.

Weight losses are undesirable release of exudates from fish flesh during processing and preservation. Since in addition to water, exudates can include water-soluble proteins, vitamins or minerals, weight losses also lead to loss of water-soluble nutrients. They involve economic losses, and thus they are an important issue for the fishing industry. Furthermore, they can negatively affect appearance or texture (Gökoğlu & Yerlikaya, 2015b; Kolbe & Kramer, 2007).

Denaturation of myofibrillar proteins, which are responsible for water-holding capacity in fish muscle (Huff-Lonergan & Lonergan, 2005), during fish processing and preservation involves an increase of weight losses during refrigerated or frozen storage (Christensen et al., 2017; Leygonie et al., 2012).

In this work, high-pressure processing has been proposed as a technology capable of decreasing weight losses of albacore. High-pressure processing (HPP) has been reported to modify muscular protein structure leading to unfolding, denaturation, aggregation, precipitation or gelation (Rastogi et al., 2007). Protein gel networks induced by pressure have been shown to improve the water retention of fish muscle (Chéret et al., 2005). However, this treatment also leads to some undesirable changes in fish quality. Negative effects of HPP treatments are strongly dependent on treatment conditions (pressure level, pressurization time and temperature) (Barba et al., 2015b). Hence, choosing the best HPP conditions (pressure, pressurization time and temperature) is essential to ensure a decrease of weight losses while retaining similar properties to non-treated fish. In spite HPP technology has been widely studied on fish products, its application for reducing weight losses while minimizing the negative effects of HPP on quality has not been studied in deep.

In the Study 1, the potential of HPP to decrease weight losses in fresh albacore (after 24 h of refrigerated storage) was evaluated using a wide pressure range (from 50 to 500 MPa) for 2 min. Weight loss of fresh albacore started to decrease after above 200 MPa. From 250 MPa weight loss was lower than in the controls, showing similar behavior to other

meat and fish products (Souza et al., 2011; Xuan et al., 2018) under similar HPP treatments. However, color and texture parameters as well as appearance started to be negatively affected by pressure from around 200 MPa, changing progressively as the pressure increased. Hence, different level pressures should be chosen depending on the specific target. The greatest weight loss reduction was observed at 500 MPa for 2 min (59.4% decrease in comparison with the control samples), but this HPP treatment resulted in sharp changes in color, texture and steaks showed a cooked-like appearance. Thus, 500 MPa for 2 min was the best HPP treatment to decrease economic losses during processing and storage, and hence it could be suitable when the product is further transformed. However, it could be rejected by consumer when it is marketed as a raw product. Between 200 and 250 MPa for 2 min there was a sharp reduction of weight loss (from 1.31 to 0.68%), which suggests that this range of pressures could be suitable to minimize weight loss in fresh albacore without affecting color, texture and appearance and thus retaining a fresh-like quality. In the Study 3 it was found that HPP treatments of 200 MPa between 0 and 4 min did not decrease or even increased weight loss of fresh albacore, whereas 200 MPa for 6 min decreased it by 36.5% with respect to the controls. Different results between samples could be explained by the influence of the time since albacore is caught until fishing vessels return to port in weight losses, as well as the physiological status of fish (Gökoğlu & Yerlikaya, 2015d). HPP treatment at 200 MPa for 6 min also significantly decreased cooking and total weight losses in fresh albacore (26.1 and 27.3% lower than the controls, respectively), although it led to several changes in color and texture of raw albacore steaks. These changes of fresh albacore were less pronounced after HPP treatment of 200 MPa for 6 min than after 250 MPa for 2 min. Furthermore, in cooked albacore steaks, color and texture parameters of HPP-treated fresh albacore at 200 MPa for 6 min were similar to those found in the controls. TBARS value of fresh albacore was not affected by HPP treatment from 50 to 500 MPa. In accordance with the results found in thawing, color and texture, all of them related to protein structure changes induced by pressure, salt-soluble protein content decreased with the intensity of the HPP treatment, showing protein denaturation and aggregation. Muscle fibers are comprised by pressure (Chéret et al., 2005; Xuan et al., 2018) and hence, intra- and intermolecular interactions between myofibrillar proteins are increased (Truong et al., 2017). Since covalent bonds are stable to pressure, protein gel network formed as a result of HPP treatment is stabilized by hydrogen bonds and, at higher pressures, also by disulfide bonds (Angsupanich et al., 1999). Hydrogen bonded gel formation leads to increased hardness

and springiness and enhances water retention (Angsupanich & Ledward, 1998; Chéret et al., 2005).

In the Study 2, HPP was proposed as a pretreatment before freezing (–20 ºC, 5 m s^{-1}) to decrease weight losses in thawed albacore just after freezing (time 0 of frozen storage) in order to assess the impact of the freezing step after HPP treatment. The freezing step was also studied in the Study 3. The freeze-thaw process increased weight losses because water from the tissue turned into ice crystals during freezing cannot be completely reabsorbed during thawing (Chevalier et al., 2000a). Denaturation and aggregation of myofibrillar proteins also contribute to the increased weight losses after the freeze-thaw process (Y. Zhang & Ertbjerg, 2019). However, the application of HPP pretreatments above 200 MPa for 4 min before freezing resulted in similar weight losses to those found in fresh albacore. Thus, these HPP pretreatments would allow the reduction of weight loss inherent to the freezing step. On the contrary, the cooking loss was higher in fresh control albacore samples than in those thawed just after freezing. This fact could be explained because part of the water lost during cooking in fresh samples could be already lost during thawing in albacore subjected to a freeze-thaw process. When considering the effect of HPP pretreatment on cooking losses, it could be said that the freeze-thaw process did not affect cooking loss regardless of the HPP treatment. Color parameters of thawed albacore just after freezing changed in the same manner than in fresh albacore: L^*, b^* and total color difference (ΔE) increased as the pressure or the pressurization time increased. The same behavior was observed in other HPP-pretreated fish species before freezing at time 0 of frozen storage (Aubourg, Torres, et al., 2013; Pita-Calvo et al., 2018b; Torres et al., 2014; Truong et al., 2015). By contrast, the freezing step offset the increasing trend of texture parameters caused by the HPP treatment in fresh albacore: whereas in fresh albacore hardness, springiness and chewiness progressively increased with the pressure and the pressurization time these trends were much less pronounced after the freezing step and even resulted in no differences with the non-treated samples. This may be attributed to ice crystals formed during freezing, which disrupt the muscle structure, leading to softening (Leygonie et al., 2012). As in fresh samples, no changes in TBARS value were found as a consequence of HPP pretreatments between 200 and 300 MPa for 0–6 min. In agreement, there was no impact of HPP pretreatment before freezing on TBARS value of barramundi (*Lates calcarifer*) samples after the freezing step (Truong et al., 2016).

In the Study 3, the effect of HPP pretreatment albacore before freezing (–20 ºC, 5 m s^{-1}) was evaluated after long-term storage (– 20 ºC, up to 12 months). It was also evaluated in the Study 5, where HPP-pretreated albacore was frozen under different freezing conditions. Thawing, cooking and total weight losses of both controls and HPP-pretreated samples remained almost stable during up to 12 months of frozen storage, since slight variations were only detected from the second month. Therefore, the freezing (–20 ºC, 5 m s^{-1}) and the frozen storage conditions (–20 ºC) applied were suitable for long-term storage of frozen albacore in terms of weight losses. Furthermore, HPP pretreatment at 200 MPa for 6 min before freezing resulted in lower thawing losses than the controls after up to 12 months of frozen storage. Nevertheless, the positive effect of HPP pretreatment on cooking and total weight losses found in thawed albacore just after freezing did not occur when it was subjected to longer frozen storage than 2 months. In the remaining quality attributes of fish, remarkable changes due to the frozen storage time were only found in $b*$ value and TBARS of raw samples, which showed an increasing trend during the frozen storage in non-treated samples. These changes were attributed to the denaturation of myofibrillar and sarcoplasmic proteins (Chouhan et al., 2015) and the release of pro-oxidants components due to the cell membrane damage caused by ice-crystals (Leygonie et al., 2012), respectively. However, HPP pretreatment at 200 MPa for 6 min allowed to minimize the increasing trends of these parameters: whereas in control samples $b*$ and TBARS value increased by 42.9 and 387.7%, respectively, in 200 MPa for 6 min samples they increased by 18.9 and 73.2%, respectively. From these results, it could be concluded that HPP pretreatment before freezing at 200 MPa for 6 min allowed to minimize weight losses inherent to the frozen storage, as well as stabilized yellowness and lipid oxidation evolution during the frozen storage. After long-term storage, color of HPP-pretreated albacore before freezing changed in the same manner than in thawed albacore just after freezing as a result of HPP pretreatment: whereas in raw albacore $L*$ and $b*$ values and total color difference increased with the intensity of the HPP pretreatment, upon cooking there were no changes between HPP-pretreated albacore and the controls.

In the Study 4, HPP was proposed as a pretreatment before the frozen storage and before thawing for assessing its potential for decreasing weight losses without affecting fish quality. In this study, HPP treatments were applied in previously frozen fish, in contrast to the Studies 2, 3 and 5, where HPP treatments were applied before freezing. Based on

the results obtained, it can be concluded that thawing losses reduction effect of HPP pretreatment at 200 MPa for 6 min was similar regardless of whether it was applied before freezing (in fresh albacore) or before frozen storage or thawing (after the freezing step) (Table 10). In addition, color, texture and salt-soluble protein content of albacore followed similar trends after the HPP pretreatment, regardless of whether it is applied before freezing, before frozen storage or before thawing. However, color and texture parameters were much more affected when HPP was applied in frozen albacore than in the fresh one. More specifically, at time 0 of frozen albacore, HPP-pretreated frozen albacores at 200 MPa for 6 min presented 51.7 and 437.7% higher $L*$ and $b*$ values than the control samples, respectively. However, when this same HPP pretreatment was applied before freezing, the increases of $L*$ and $b*$ values were around 30 and 150% with respect to their controls. Regarding the texture, when HPP treatment at 200 MPa for 6 min was applied in frozen albacore at time 0 of frozen albacore, hardness and increased by 39.8%, whereas when it was applied before freezing, this increase was lower (around 15%) and even, no differences between HPP-pretreated albacore at 200 MPa for 6 min and the controls were found in the Study 2. Thus, sample temperature during HPP treatments could affect color and texture changes induced by pressure. Accordingly, Schubring et al. (2003) suggested that, under the same pressure, color was more affected by HPP treatments at lower temperatures.

Since freezing rate is the main factor that determinates weight losses in frozen meat products (Y. H. B. Kim et al., 2015), in the Study 5 HPP-pretreated albacore before freezing was subjected to different freezing conditions (–20 and –50 ºC; 1 m s^{-1} and 5 m s^{-1}) in order to evaluate the suitability of HPP pretreatments to decrease weight losses after long-term storage (–20 ºC, up to 9 months) in albacore frozen at different freezing rates (characteristic freezing times ranging from 94.7 ± 7.9 to 10.9 ± 3.5 min). Thawing loss was independent of the freezing rate in both control and HPP-pretreated samples during up to 9 months of frozen storage. In general terms, the freezing rate did not affect color, texture and protein denaturation and aggregation in HPP-pretreated albacore, since only hardness showed some differences as a result of the freezing conditions. Although freezing conditions have not been previously considered on previous works on HPP pretreatment before freezing, comparison between different frozen storage temperatures (–10, –18 and –30 ºC) after HPP pretreatment has been recently carried out in hake (Carrera et al., 2020). These authors concluded that the positive effect of HPP

pretreatment on frozen fish quality was higher as the frozen temperature increased (Carrera et al., 2020).

Table 10. Effect of HPP pretreatments at 200 MPa for 6 min on albacore quality parameters.

	HPP pretreatments (200 MPa for 6 min)		
	Before freezing	Before frozen storage	Before thawing
Thawing loss	Reduction after 0, 2, 6 and 12 months of frozen storage	Reduction after 15 and 45 days of frozen storage	Reduction after 15 and 45 days of frozen storage
Color	Increase in $L*$ and $b*$ value after 0, 2, 6 and 12 months of frozen storage	Increase in $L*$ and $b*$ value after 15 and 45 days of frozen storage	Increase in $L*$ and $b*$ value after 15 and 45 days of frozen storage
Texture	Increase in hardness after 0 months of frozen storage. No changes from 2 months of frozen storage. Increase in adhesiveness and springiness after 0, 2, 6 and 12 months of frozen storage. Increase in chewiness after 0 and 12 months of frozen storage.	No changes in hardness. Increase in adhesiveness, springiness and chewiness after 15 and 45 days of frozen storage.	Increase in hardness, springiness and chewiness after 15 and 45 days of frozen storage. Increase in adhesiveness after 15 days of frozen storage.
Salt-soluble protein content	Decrease after 0, 2, 6 and 12 months of frozen storage	Decrease after 15 and 45 days of frozen storage	Decrease after 15 and 45 days of frozen storage
TBARS value	No changes after 0 months of frozen storage. Decrease from 6 months of frozen storage.	No changes	Increase after 15 and 45 days of frozen storage

HPP pretreatment at 200 MPa for 6 min did not lead to consistent results in terms of cooking and total weight losses. Overall, cooking losses were much higher than thawing losses. Thus, the water retained by protein gelation induced by the HPP treatment cannot be always maintained during cooking. It is known that cooking losses are highly

dependent on the cooking method and the cooking temperature (Pérez Chabela & Mateo-Oyague, 2005). It would be interesting to evaluate whether less intensive heat treatments than those evaluated in the present work could also lead to a lower cooking loss after HPP treatment at 200 MPa for 6 min.

In view of the results obtained, it has been shown the potential of HPP treatment at 200 MPa for 6 min for decreasing weight losses in fresh albacore as well as thawing losses in the frozen one. After shorter frozen storage times (≤ 2 months), there was not an inhibitory effect of HPP pretreatments on lipid oxidation regardless whether it is applied before freezing, before frozen storage or before thawing (Table 10). Hence, longer frozen storage times than 6 months could be necessary to induce the decrease of the activity of enzymes involved in lipid hydrolysis and lipid oxidation.

The present work shows that high-pressure pretreatments may be a suitable technology to enhance the quality of frozen albacore.

5.CONCLUSIONS

Conclusions

The main conclusions of this work, which contribute to a better knowledge of the effect of high-pressure (HPP) treatment on frozen fish, are listed below:

1. HPP led to an improvement of water retention in fresh albacore from 200 MPa for 2 min. However, HPP also involved negative changes in color, appearance and texture, which increased progressively with the intensity of the treatment and distinguishable around 200–250 MPa for 2 min.

2. The impact of HPP on fish quality was highly dependent on the treatment conditions (pressure level and pressurization time). Therefore, these HPP conditions must be selected according to the ulterior use of the fish (fresh, frozen, cooked, canned, transformed, etc.).

3. HPP pretreatment at 200 MPa during 4–6 min before freezing allowed the reduction of weight losses during up to 12 months of frozen storage, maintaining similar values to those observed in fresh non-treated fish. Hence, HPP pretreatment could offset the weight losses related to the freeze-thaw process after long-term storage.

4. In HPP-pretreated albacore before freezing, color changed in the same manner as in fresh fish (*L**, *b** and total difference increase progressively as the intensity of the treatment increases). However, the texture behavior was different than in fresh fish, since the hardness increase observed in fresh albacore did not take place in the frozen one after longer storage times (from the second month).

5. There was not a consistent effect of HPP pretreatments on cooking and total weight losses. However, weight losses induced by heat treatment were much higher in all cases than those induced by pressure. It is necessary to carry out further research on the effect of different heat treatments on weight losses of HPP-pretreated albacore.

6. When raw HPP-treated albacore was subsequently subjected to a heat treatment, pressure-mediated changes in color and appearance were no longer evident. Therefore, HPP may be an interesting technology to improve the fish quality when it is intended for further processing, such as cooking or canning.

7. Excluding lipid oxidation, most quality parameters (color, texture) remained stable throughout long-term frozen storage. Lipid oxidation development was inhibited by the application of HPP pretreatments after long-term frozen storage (from 6 months).

8. Color, texture and salt-soluble protein content of albacore followed similar trends after the HPP pretreatments. However, the HPP pretreatment before freezing had less impact on color and texture of albacore steaks than HPP pretreatments before storage or before thawing.

9. Hardness was the only parameter that changed as a result of the different freezing conditions. HPP pretreatment at 200 MPa for 6 min exerted much more impact on the fish quality than the freezing conditions.

10. HPP pretreatments are a useful technology for decreasing weight losses caused by processing and storage with a minimal impact on color, appearance and texture, and thus retaining similar quality properties to non-treated albacore.

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ANNEX

Publications derived from this Thesis:

1. Cartagena, L., Puértolas, E., & Martínez de Marañón, I. (2019). **High-pressure Processing (HPP) for decreasing weight loss of fresh albacore (*Thunnus alalunga*) steaks.** *Food and Bioprocess Technology*, *12*, 2074–2084. https://doi.org/10.1007/s11947-019-02369-w.

2. Cartagena, L., Puértolas, E., & Martínez de Marañón, I. (2021). **High-pressure pretreatment in albacore (*Thunnus alalunga)* for reducing freeze-driven weight losses with minimal quality changes**. *Journal of the Science of Food and Agriculture, 101*, 2074-2711. https://doi.org/10.1002/jsfa.10895.

3. Cartagena, L., Puértolas, E., & Martínez de Marañón, I. (2020). **Evolution of quality parameters of high-pressure processing (HPP) pretreated albacore (*Thunnus alalunga*) during long-term frozen storage**. *Innovative Food Science and Emerging Technologies*, *62*, 102334. https://doi.org/10.1016/j.ifset.2020.102334.

4. Cartagena, L., Puértolas, E. & Martínez de Marañón, I. (2020). **Application of high-pressure processing after freezing (before frozen storage) or before thawing in frozen albacore tuna (*Thunnus alalunga*)**. *Food and Bioprocess Technology*, *13*, 1791–1800. https://doi.org/10.1007/s11947-020-02523-9.

5. Cartagena, L., Puértolas, E. & Martínez de Marañón, I. (2021). **Impact of different air blast freezing conditions on the physicochemical quality of albacore (*Thunnus alalunga*) pretreated by high pressure processing**. *LWT*, *145*, 111538. https://doi.org/10.1016/j.lwt.2021.111538.

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