Food Research International 112 (2018) 233–240

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Effect of high-pressure processing pretreatment on the physical properties and colour assessment of frozen European hake (*Merluccius merluccius*) during long term storage

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ARTICLE INFO

Keywords:
Merluccius merluccius
High pressure processing
Frozen storage
Physical properties
Quality enhancement

ABSTRACT

Fish freshness is lost by autolytic degradation produced by endogenous enzymes. Frozen storage is one of the most used methods to preserve fish properties. However, protein denaturation has shown to be a major problem for frozen European hake (*Merluccius merluccius*), leading to texture losses and off-odour development. The aim of this work was to study the changes produced by high-pressure processing (HPP) before freezing on quality of frozen European hake stored at −21 °C for 12 months. The effect of HPP (150–450 MPa) on mechanical properties and expresible water was evaluated in raw and cooked fish samples. The effect on colour (L*, a* and b*) was assessed only in raw fish. Results showed that HPP before freezing is beneficial to maintain expresible water in good levels up to 6 months. The luminosity significantly increased with pressure level. Textural profile of raw samples showed that HPP increased hardness, adhesiveness and springiness of frozen hake. Cooked samples were also affected by HPP, being the best results obtained at 300 MPa for 6 months of frozen storage. Overall, results showed that HPP improves the quality of frozen hake.

1. Introduction

High pressure processing (HPP) is a non-thermal technique of growing interest for the processing and preservation of food. HPP is incresingly used commercially to pasteurize food products such as fruit juices, meat products, and seafood (Medina-Meza, Barnaba, & Barbosa-Cánovas, 2014). It eliminates food pathogens at room temperature extending the shelf life through the cold chain (Huang, Wu, Lu, Shyu, & Wang, 2017). The effect of high pressure on microorganisms and proteins/enzymes was observed to be similar to that of high temperature. However, when compared to traditional thermal pasteurization, HPP processing has the advantage that it retains better the organoleptic properties and nutritional value of food (Křížek, Matějková, Vácha, & Dadáková, 2014; Medina-Meza et al., 2014). Moreover, HPP is also an interesting technology for food industry due to its ability to modify functional properties and create new textures (Chapleau & De Lamballerie-Anton, 2003).

HPP is used on several types of seafood though its potential for extending the shelf life of fresh fish has not been fully exploited (Rode & Hovda, 2016). HPP inactivates to different levels a wide variety of enzymes which produce fish spoilage, thus reducing their activity during storage. The decreasing of endogenous enzymes activity was observed in pelagic fish species such as Atlantic mackerel (*S. scombrus*) and horse mackerel (*Trachurus trachurus*) when HPP pre-treatment before freezing and frozen storage was applied (Fidalgo, Saraiva, Aubourg, Vázquez, & Torres, 2014a; Fidalgo, Saraiva, Aubourg, Vázquez, & Torres, 2014b). Reduction of lipid deterioration was also observed in these fish species (Torres, Vázquez, Saraiva, Gallardo, & Aubourg, 2013; Vázquez, Torres, Gallardo, Saraiva, & Aubourg, 2013) and more recently in sardine (*Sardina pilchardus*) (Méndez et al., 2017).

Different pressure levels and holding times can denature myofibrillar proteins and produce changes in protein functionality, visual appearance, and mechanical properties of fish muscle to different extents (Uresti, Velazquez, Vázquez, Ramírez, & Torres, 2005). For example, in sea bass (*Dicentrarchus labrax*) fillets treated at 250–400 MPa for 5–30 min, myofibrillar proteins with molecular weights below 30 kDa increased, whereas those with lower isolectric point values decreased. Furthermore, fish muscle become whitish, pH increased, and

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https://doi.org/10.1016/j.foodres.2018.06.042
Received 14 May 2018; Received in revised form 19 June 2018; Accepted 20 June 2018
Available online 21 June 2018
0963-9969/

microbiological load and water holding capacity decreased. Changes in the protein profiles might explain the effect of HPP on fish muscle physical properties (Teixeira et al., 2014). In cold smoked salmon (*Salmo salar*), high pressures (600–900 MPa) and holding times (30 and 60 s) produced a considerable shrinkage of myofibrils resulting in increased space between them (Gudbjornsdottir, Jonsson, Hafsteinsson, & Heinz, 2010).

Treatment at 300 MPa for 15 min effectively reduced susceptibility to oxidation in Atlantic salmon (*Salmo salar*) and led to total microbial reduction (Yagiz et al., 2009). Moreover, HPP (150 or 300 MPa for 15 min) did not produce changes in terms of total saturated, monoenes, n-3 PUFA and n-6 PUFA polyunsaturated fatty acid profile. The effect of pressure level (135–200 MPa) on sensory and physical properties of chilled coho Salmon (*Oncorhynchus kisutch*) was studied by several researchers (Yagiz et al., 2009). According to odour (rancid, putrid), texture (elasticity, gaping, firmness), and colour (L* value) attributtes, 135 MPa showed to be the most effective treatment (Aubourg, Rodríguez, Sierra, Tabilo-Munizaga, & Pérez-Won, 2013). Higher pressure levels reduced the microbial load but caused an increase in the lipid oxidation (Aubourg, Rodríguez, et al., 2013) and marked damage of sarcoplasmic proteins fraction (Ortea, Rodríguez, Tabilo-Munizaga, Pérez-Won, & Aubourg, 2010).

A compative study of effect of HPP on salmon (*Salmo salar*), cod (*Gadus morhua*), and mackerel (*Scomber scombrus*) on several quality paramenters (microbiological load, pH, lipid oxidation, and acid phosfatase activity), revealed that HPP at 200 or 500 MPa induced different changes on these parameters depending on the type of fish species (Rode & Hovda, 2016). A species-dependent effect was also reported for the effect of HPP followed by freezing and frozen storage on the functional and sensory properties of Atlantic mackerel (*S. scombrus*) and horse mackerel (*T. trachurus*) (Aubourg, Torres, Saraiva, Guerra-Rodríguez, & Vázquez, 2013; Torres, Saraiva, Guerra-Rodríguez, Aubourg, & Vázquez, 2014), what can be due to differences in fat composition.

Some studies showed that the application of HPP before refrigerated storage could noticeably reduce the contents of biogenic amines in vacuum-packed pike (*Esox licius*) (Křížek et al., 2014), smoked cod (*Gadus morhua*) (Montiel, De Alba, Bravo, Gaya, & Medina, 2012) and rainbow trout (*Oncorhynchus mykiss*) (Matějková, Křížek, Vácha, & Dadáková, 2013).

European hake (*Merluccius merluccius*) is a gadiform species that has high nutritional value and healthy properties, but frozen storage of this fish is very limited due the protein denaturation that leads to texture losses and off-odour development. Different pathways have been proposed to explain this degradation process such as partial dehydration of proteins during freezing due to the formation of ice crystals, interaction of proteins with formaldehyde and lipids, and modification of the environment in the liquid phase surrounding proteins caused by the concentration of salts (Vázquez, Fidalgo, Saraiva, & Aubourg, 2018). Consequently, it is of interest to explore technological treatments that can overcome such drawbacks in order to increase fish shelf life and, accordingly, its trading value.

Using a relatively high temperature of frozen storage (−10 °C), a previous accelerated frozen storage study (5 months) was carried out by our group to assess the effect of HPP pre-treatment before freezing and frozen storage on mechanical and physical properties of frozen small European hake (*Merluccius merluccius*) (Pita-Calvo, Guerra-Rodríguez, Saraiva, Aubourg, & Vázquez, 2017), allowing to gain insights about the effect of HPP in a shorter experimental time period. The results obtained were very promising, but extrapolation to a more usual frozen storage temperature (−21 °C) used commercially is not straightforward and it is necessary to study the effect of HPP at this temperature. Therefore, the aim of this work was to study the changes produced by HPP pre-treatments on quality of frozen European hake stored at −21 °C for 12 month, studying mechanical properties and expresible water in raw and cooked fish and colour in raw fish.

2. Materials and methods

2.1. Raw fish, processing, storage, and sampling

European hake (*M. merluccius*) was purchased at the Vigo harbour (Galicia, northwest Spain). Fish was transported to “Plataforma Tecnológica Multidisciplinar Alta Pressão” (University of Aveiro, Portugal) in a refrigerated truck. Samples packed in polyethylene bags were vacuum sealed at 400 mbar. The length of the specimens varied between 27.5 and 29.5 cm and its weight between 180 and 205 g.

Fish processing was carried out in a HPP equipment (55-L-WAVE 6000/55HT; Hyperbaric, Spain). The pressure levels tested were 150, 169.27, 300, 430.73, and 450 MPa. These pressure levels were reached at 50, 56.42, 100, 143.58, and 150 s, respectively, using a rate of 3 MPa s^{-1}. The holding time of the pressure was 2 min and the decompression time < 3 s. Processing was carried out at room temperature using pressurizing water at 20 °C. After HPP treatment, hake specimens were frozen at −21 °C for 48 h and frozen storage at this temperature as described earlier (Aubourg, Torres, et al., 2013). Samples without HPP processing (control samples) were also tested; sampling was carried out for 12 months of frozen storage following the experimental design showed below. Cooked fish samples were obtained heating at oven at 200 °C for 15 min.

2.2. Expressible water and colour parameters

The expressible water (E_w) values were determined in raw and cooked samples following the procedure described in the literature (Martelo-Vidal, Guerra-Rodríguez, Pita-Calvo, & Vázquez, 2016). Four analyses were carried out for each treatment from several samples.

Colour was determined in the raw fish flesh as described previously (Rocio M. Uresti, López-Arias, Ramírez, & Vázquez, 2003). Values of L*, a*, and b* were determined based on illuminate C and the 2° standard observer in reflection mode using a colorimeter ColorStriker (Mathai, Hannover, Germany). Nine measures were obtained for each treatment from several samples.

2.3. Texture profile analysis (TPA)

Texture profile for each treatment was analyzed in raw and cooked fish samples. A texturometer (TA-XTplus, Stable Micro System, UK) equipped with an aluminium cylindrical probe (P/50–50 mm diameter) was used. Samples were cut into small pieces (2 × 2 × 1.5 cm) being compressed to 75% of their original height using a compression speed of 60 mm/min. For each treatment, five textural parameters (hardness, adhesiveness, springiness, cohesiveness, and chewiness) were evaluated. Ten analyses using several samples were carried out for each treatment (Cortez-Vega, Fonseca, Feisther, Silva, & Prenticea, 2013; da Silva, Lourenço, & Pena, 2017; Palmeira, Mársico, Monteiro, Lemos, & Conte Junior, 2016).

2.4. Statistical analysis

The experimental design was statistically analyzed by the Design Expert® 7.1.1 software (Stat-Ease, Inc., MN, USA). The set of experiments followed a central composite design (CCD) which handles three groups of design points: (a) two-level factorial design points; (b) axial or “star” points, and (c) center points. This design uses five levels of the independent variables with desirable statistical properties. In this case, in order to study the range of pressures from 150 to 450 MPa, the design gave the following levels: 150, 169.27, 300, 430.73, and 450 MPa. The range of frozen time was from 0 to 12 months. The design gave the following levels: 0, 0.778, 6, 11.232, and 12 months (0, 23, 180, 337 and 360 days). The models used and the statistical approach are described earlier (Méndez et al., 2017). Cook's distance was applied the detect outliers (Cook, 1977).