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Effect of high-pressure pre-treatments on enzymatic activities of Atlantic mackerel (*Scomber scombrus*) during frozen storage

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ABSTRACT

In this work, we studied the effect of high-pressure processing (HPP) pre-treatments (150, 300 and 450 MPa for 0, 2.5 and 5 min) on key enzyme activities in Atlantic mackerel (*Scomber scombrus*) during frozen storage (3 months of accelerated storage, −10 °C). Except for a minor decrease from treatment at 450 MPa, no substantial effect on acid phosphatase activity was observed. In contrast, cathepsins B and D and lipase were affected by HPP and frozen storage. Increasing the pressure reduced cathepsin B and lipase activities. Generally, increasing the holding time at 150 MPa increased the activity of cathepsin B and lipase (except at month 1), while increasing the holding time at 300 MPa reduced the lipase activity, and no effect was observed at 450 MPa. Overall, cathepsin D activity increased with frozen storage time and for treatments at 300 MPa but decreased at 450 MPa. This work provides novel information of HPP pre-treatments application, lowering enzyme activity during frozen storage of Atlantic mackerel.

Industrial relevance: The activity of fish endogenous enzymes has an important role in its deterioration during frozen storage, limiting its commercialisation. This research presents valuable information concerning the employment of high-pressure processing pre-treatments to reduce deteriorative enzymatic activities during the subsequent frozen storage of Atlantic mackerel (*Scomber scombrus*). High-pressure processing pre-treatments can so be of interest for fish processors to enhance the frozen storage of these fish species and possibly of others.

1. Introduction

Small pelagic fish species constitute food products of great economic importance in many European countries (Huidobro, Montero, Tejada, Colmenero, & Borderías, 1990). Although recognized as a healthy food, mackerel (*Scomber scombrus*), a species abundant in the northeast Atlantic, remains underutilized because of its short chilled shelf life (up to 9–10 days) (Sanjuas-Rey, Gallardo, Barros-Velazquez, & Acubourg, 2012). Mackerel and other fresh fish are extremely perishable when compared to other food commodities, and thus freezing and frozen storage is most often used for their preservation. However, quality is lost during frozen storage due to texture, flavour and colour deterioration (Matsumoto, 1979). Deterioration of fish during frozen storage depends on many factors including fish species, storage temperature, time and endogenous enzymatic activity. According to Burgaard and Jørgensen (2011), frozen storage temperature did not seem to affect cathepsin D activity in trout. However, Nilsson and Ekstrand (1995) observed that frozen storage temperature affects lysosomal membrane integrity resulting in increased lysosomal enzymes leakage and, thus, increased β-*N*-acetylglucosaminidase activity in trout samples stored at −18 °C as compared to −40 °C. The release of lysosomal lipases in trout decreased during frozen storage (Geromel & Montgomery, 1980). At the same time, lipase activity is the principal cause of hydrolysis and formation of free fatty acids during frozen fish storage (Gallardo, Aubourg, & Perezmartin, 1989).

HPP is a non-thermal technique for food preservation that efficiently inactivates vegetative microorganisms while retaining high quality levels (Mújica-Paz, Valdez-Fragoso, Tonello Samson, Welti-Chanes, & Torres, 2011). HPP is applied commercially in the 100- to 700-MPa range, allowing most foods to be preserved with minimal effect on taste, texture or nutritional characteristics (Balasubramaniam, Farkas, & Turek, 2008; Mota, Lopes, Delgadillo, & Saraiva, 2013; Ramirez, Saraiva, Lamela, & Torres, 2009; Yordanov & Angelova, 2010). HPP was shown to inactivate enzymes by disrupting the bonds that determine the secondary, tertiary and quaternary conformations without affecting the covalent bonds in the primary structure. HPP at 100–300 MPa (up to 30 min), applied on enzyme extracts from bluefish and sheephead, decreased the enzyme activity, especially of cathepsin C, collagenase, chymotrypsin and trypsin-like enzymes (Ashie & Simpson, 1996). However, pressures up to 500 MPa increased the cathepsins B, H and L activities in sea bass muscle (Cheret, Delbarre-Ladrat, De Lamballerie-Anton,

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& Verrez-Bagnis, 2005) and decreased the calpain activity while evolving differently during subsequent refrigerated storage (Cheret, Delbarre-Ladrat, Verrez-Bagnis, & De Lamballerie, 2007; Cheret, Hernandez-Andres, Delbarre-Ladrat, de Lamballerie, & Verrez-Bagnis, 2006). Teixeira et al. (2013) obtained similar results using sea bass muscle, with higher activity reduction observed at about 400 MPa for acid phosphatase, cathepsin D and calpain.

Recent previous work demonstrated an inhibition of lipid hydrolysis (Vázquez, Torres, Gallardo, Saraiva, & Aubourg, 2012) and improved functional and sensory properties (Aubourg, Torres, Saraiva, Guerra-Rodríguez, & Vázquez, 2013) in Atlantic mackerel (*S. scombrus*) samples subjected to HPP pre-treatments before freezing and subsequent frozen storage. However, there is limited information on the effect of HPP pre-treatments on the activity of endogenous enzymes during frozen storage of fish. The aim of this work was to study the effect of HPP pre-treatments on the activity of several quality degrading enzymes (acid phosphatase, cathepsins B and D and lipase) during frozen storage of Atlantic mackerel.

2. Materials and methods

2.1. Chemicals

The enzyme substrates *p*-nitrophenylphosphate (*p*-NPP), Z-arginine-arginine-7-amido-4-methylcoumarin hydrochloride (Z-Arg-Arg-7-AMC HCl, #C5429), hemoglobin from bovine blood and olive oil were purchased from Sigma-Aldrich (Steinheim, Germany); the chemicals sodium dodecyl sulfate (SDS), trichloroacetic acid (TCA), acetic acid, trizma hydrochloride (Tris–HCl), dithiothreitol (DTT), 2-bis-(2-hydroxyethyl) amino-2-(hydroxymethyl)-1,3-propanediol (Bis-Tris), ethylenediaminetetraacetic acid (EDTA), *p*-nitrophenol (*p*-NP), thymolphtalein, sodium hydroxide (NaOH), citric acid, trisodium citrate and L-tyrosine were also obtained from Sigma-Aldrich (Steinheim, Germany); other chemicals, such as potassium hydroxide (KOH) and sodium acetate, were acquired from Panreac Quimica S.L.U. (Barcelona, Spain); and the ethanol had a purity grade of 99%.

2.2. Preparation, processing and storage of sample

Atlantic mackerel (*S. scombrus*) caught near the Bask coast in Northern Spain (Ondarroa harbor, Bizkaia, Spain) were transported under refrigeration to the AZTI Tecnalia (Derio, Spain) pilot plant for HPP treatment within 6 hours after catch. Whole Atlantic mackerel (28–33 cm and 230–280 g range) individuals were placed in flexible polyethylene bags (three individuals per bag) and vacuum sealed at 400 mbar.

Whole fish were HPP-treated in a 55-L high-pressure unit (WAVE 6000/55HT; NC Hyperbaric, Burgos, Spain) according to the following experimental design: T1 (450 MPa, 0 min), T2 (450 MPa, 2.5 min), T3 (450 MPa, 5 min), T4 (300 MPa, 0 min), T5 (300 MPa, 2.5 min), T6 (300 MPa, 2.5 min), T7 (300 MPa, 2.5 min), T8 (300 MPa, 5 min), T9 (150 MPa, 0 min), T10 (150 MPa, 2.5 min), T11 (150 MPa, 2.5 min) and T12 (150 MPa, 5 min). The 0-min holding time samples were carried out to study the effect of just the pressure come up and depressurising time. Non-pressure-treated samples (T0, untreated control samples) were also studied. The pressurising medium was water applied at 3 MPa/s, yielding come up times of 50, 100 and 150 s for treatments at 150, 300 and 450 MPa, respectively, while decompression time took less than 3 s. Pressurising water was cooled down to maintain room temperature (20 °C) conditions during HPP treatment. HPP-treated samples were kept frozen at −20 °C for 48 h before storage at −10 °C and sampling after 0, 1 and 3 months. A storage temperature (−10 °C) higher than that employed commercially (−18 °C) was chosen to accelerate the effect of storage time.

2.3. Enzymatic activity

2.3.1. Preparation of enzymatic extract

The enzymatic extract was prepared as described by Lakshmanan, Miskin, and Piggott (2005). Fish samples (10 g) of pooled fish muscle from each of three individuals (control or HPP-treated samples) were homogenized with 50 mL ice-cold distilled water for 2 min, using an IKA Ultra-Turrax T25 homogenizer (2 min, 8000 rpm; Janke & Kunkel, Deutschland, Germany). The homogenate was kept in ice for 30 min with occasional stirring. After 30 min, it was centrifuged at 4 °C for 20 min at 14,600×*g* (Laboratory Centrifuge 3 K30, Sigma, Osterode, Germany). The supernatant was filtered through a Whatman no. 1 filter and stored at −20 °C prior to enzymatic activity quantifications.

2.3.2. Acid phosphatase activity

Acid phosphatase activity was assayed with *p*-NPP as substrate following the methodology described by Ohmori, Shigehisa, Taji, and Hayashi (1992), with minor modifications. Extracts (0.250 mL) and substrate solution (0.225 mL) of 4 mM *p*-NPP in 0.1 mM acetate buffer and 1 mM EDTA, at pH 5.5, were incubated at 37 °C for 15 min. The reaction was stopped by adding 1 mL 100 mM KOH, and the *p*-NP released was measured at 400 nm (Perkin-Elmer Instruments Lambda 35 UV/vis spectrometer, Massachusetts, USA). Acid phosphatase activity was expressed as nmol *p*-NP/min/g of fresh fish. Three replicates were performed for each treatment.

2.3.3. Cathepsins activity

2.3.3.1. Cathepsin B. Cathepsin B activity was assayed by the methodology described by Lakshmanan et al. (2005). Enzyme extract (0.1 mL) and substrate solution (0.1 mL), containing 0.0625 mM of Z-Arg-Arg-7-AMC HCl in 100 mM Bis-Tris buffer with 20 mM EDTA and 4 mM DTT, at pH 6.5, were incubated at 37 °C for 5 min. The reaction was stopped by adding 1 mL 3% SDS (w/v) in 50 mM Bis-Tris (pH 7.0), and the AMC liberated was measured by fluorescence (excitation: 360 nm, emission: 460 nm; Hitachi F2000 fluorescence spectrophotometer, Tokyo, Japan). Cathepsin B activity was expressed as fluorescence units (FU)/min/g of fresh fish. Three replicates were performed for each treatment.

2.3.3.2. Cathepsin D. Cathepsin D activity was assayed as described by Buckow, Truong, and Versteeg (2010), with small modifications. Enzyme extract (0.2 mL) was mixed with 0.6 mL of substrate solution, 2% denatured hemoglobin (w/v) in 200 mM citrate buffer (pH 3.7) and incubated for 3 h at 37 °C. The reaction was stopped by the addition of 0.6 mL 10% TCA (w/v). After vigorous stirring, the precipitate was removed by centrifugation (18,000×*g* for 15 min; Elmi Micro Centrifuge CM-50, Porvoo, Finland) and the soluble peptides measured at 280 nm (Perkin-Elmer Instruments Lambda 35 UV/vis spectrometer, Massachusetts, USA). Cathepsin D activity was expressed as μg tyrosine/min/g of fresh fish. Three replicates were performed for each treatment.

2.3.4. Lipase

Lipase activity was assayed with olive oil as substrate following procedures recommended by the titrimetric enzymatic assay supplier (Sigma-Aldrich, 1999). Enzyme extract (1 mL) was mixed with the substrate solution (1.50 mL of olive oil, 1.25 mL of distilled water and 0.50 mL of 200 mM Tris–HCl buffer, pH 7.7) and incubated at 37 °C for 24 h. The reaction was stopped by adding 2 mL 95% ethanol (v/v) and the liberated free fatty acids (FFA) were titrated against 25 mM NaOH using thymolphtalein as indicator. Lipase activity was expressed as μmol FFA/min/g of fresh fish. Three replicates were performed for each treatment.