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Stress relaxation behaviour and structural changes of muscle tissues from Gilthead Sea Bream (*Sparus aurata* L.) following high pressure treatment

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ABSTRACT

Sea Bream muscle tissue was subjected to different high pressure treatments, and rheological changes were monitored during storage by means of the stress relaxation test. The best fit was obtained by application of the three term Maxwell exponential model, followed by the Nussinovich model. The application of 300 and 400 MPa pressures appeared to enable preservation of elasticity and stiffness of fish muscle during storage, compared to untreated samples. On the contrary, samples treated at 200 MPa underwent a decrease in elasticity during storage. The water holding capacity of dorsal muscle was also assessed, and it was found to decrease with increasing pressures. Immunoblot studies performed on the main structural proteins revealed that a pronounced time-dependent degradation of desmin, observed in untreated samples, could be prevented by treatment at 400 MPa. Taken together, our results strongly suggest that high pressure treatments inactivate degrading enzymes acting on proteins that are related to tissue integrity preservation, texture quality, and water holding capacity.

1. Introduction

Quality of fish as a food has been defined as "a combination of such characteristics as wholesomeness, integrity, and freshness" (Martin, 1988). Freshness is one of the most important factors for evaluating fish quality since it can be stated directly through appearance, texture and taste.

High pressure processing is a preservation technology which allows decontamination of foods at low or moderate temperature, with the major advantage of extending shelf life through microbial inactivation with minimum loss of quality (Cheftel and Culioli, 1997). Studies on the application of this technology to fish have been focused on the effects of treatments on muscle structural proteins, on oxidative stability (Chéret et al., 2006; Sequeira-Munoz et al., 2006), inactivation of proteolytic enzymes related to quality (Ashie and Simpson, 1996), changes in physicochemical parameters (Lakshmanan et al., 2007; Chéret et al., 2005), and high pressure assisted thawing (Rouillé et al., 2002; Schubring et al., 2003). Texture Profile Analysis (TPA) (Angsupanich and Ledward, 1998; Chéret et al., 2005) and puncture test (Ashie and Simpson, 1996) have been used to evaluate the textural changes in fish following high pressure treatment. Nevertheless, both methods present drawbacks, since they are destructive and time consuming. Therefore, non-destructive methods are needed in order to evaluate changes introduced in fish muscle by high pressure treatments. In this respect, the stress relaxation test can be used for quality assurance of high pressure treated fish, being fast, robust, simple, and non-destructive (Herrero et al., 2004).

The stress relaxation test (or step strain test) is one of the most important evaluation tools used for determining viscoelastic properties of materials. In a stress relaxation test, the sample is given an instantaneous strain and the stress required to maintain the deformation is observed as a function of time. A generalised Maxwell model is frequently used to interpret stress relaxation data (Herrero et al., 2004; Jain et al., 2007; Ma et al., 1996). The model consists of n Maxwell elements and a free spring in parallel, each element consisting in 1 spring and 1 damper in series. (Steffe, 1992). The generalised Maxwell model can be written as follows:

$$\sigma(t) = \sum_{i=1}^{n} Ci(e^{-(t/\tau_i)}) + \sigma_e \quad (1)$$

where σ is the stress (N) at a given time, t (seconds), Ci are stress relaxation constants (N), σ_e is the equilibrium stress (N), τ_i (seconds) are the relaxation times of the Maxwell elements.

A simplified version of the Maxwell model is the model of Nussinovich (Nussinovich et al., 1989). In this model, the relaxation times of Maxwell elements (τ_i) are given as constants. A three elements Nussinovich model can be represented as follows:

$$\sigma(t) = A_0(A_1e^{(-t/100)} + A_2e^{(-t/10)} + A_3e^{(-t/1)}) \quad (2)$$

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where σ is the stress (N) at a given time, t (seconds), A_0, A_1, A_2, A_3 are constants (N). The model allows to obtain relaxation data in short terms, avoiding the reaching of an equilibrium stress to end the test.

A third model (Peleg, 1980) is used for linear fitting of the stress relaxation data:

$$\frac{\sigma_0 t}{\sigma_0 - \sigma} = k_1 + k_2 t \quad (3)$$

where σ_0 is the initial stress (N), σ (N) is the decaying stress at a given time, t (seconds), K_1, and K_2 are dimensionless constants. $1/K_1$ gives the initial relaxation rate, while $1/K_2$ represents the proportion of relaxed initial force and gives a measure of the "solidity" of the material (Gamero et al., 1993). $1 - 1/K_2$ is the magnitude of the asymptotic residual modulus and gives a measure of the stress that remains unrelaxed (Nussinovich et al., 1989).

Textural changes are related to evolution of muscle constituents, primarily proteins, with the endogenous calpain system playing a major role in proteolysis of muscle proteins under post-mortem conditions (Lonergan et al., 2001; Maddock et al., 2005). High pressure treatments may affect muscle constituents, primarily large molecules as proteins. Covalent bonds in proteins are not affected by high pressure while ionic and hydrogen bonds and then tertiary structure can change noticeably. Particularly, modifications in enzymes structure and compartmentation may be of chief importance because of their role in post-mortem proteolysis. Also, the ability of muscle to retain water is strictly related to the post-mortem events such as pH decline, proteolysis and protein oxidation (Huff-Lonergan et al., 1996), and it is very important in fish both from a quality and, consequently, commercial point of view. Aim of the present work was to evaluate the changes induced by high pressure treatments in sea bream muscle, by relating modifications in texture and water holding capacity to changes in myofibrillar and cytoskeletal proteins. The rheological properties of high pressure treated farmed headed/gutted Gilthead Sea Bream during chilled storage were assessed by stress relaxation test. The data generated were fitted with three different models, in order to allow the best possible interpretation of the relaxation curves obtained. Water Holding Capacity was evaluated by centrifugal methods. The status of structural proteins was investigated by means of electrophoresis and western immunoblotting.

2. Materials and methods

2.1. Samples and high pressure treatment

Sixty farmed Sea Breams were obtained from an aquaculture plant located in South-western Sardinia (Italy). After capture, fish (18 months of age, average length 26 cm, average weight 262.3 g) were kept in melting ice, transported to the laboratories, and stored in ice for 24 h. Then, fish were manually headed, gutted, washed in cold water (+3 °C) and vacuum sealed individually in plastic bags (co-extruded PA/PE-20/70; O_2 transmission rate: 30–50 cm^3/m^2–24 h-atm; water vapour transmission rate: 2.6 g/m^2-atm; CO_2 transmission rate 150 cm^3/m^2-24 h-atm; N_2 transmission rate 10 cm^3/m^2-24 h-atm, at 23 °C and 50% RH). Samples were divided in four groups. One group (not pressurised) was kept as a control in chilled conditions (+3 °C), while the remaining were subsequently pressurised. Prior to high pressure treatments, packed samples were kept on an ice bath (0–2 °C) in order to prevent adverse effects induced by temperature. Treatments were carried out with a Pilot scale HPP410100 Isostatic Press (Flow Autoclave Systems Inc, Columbus, Ohio, USA). Internal diameter of the vessel was 100 mm, inside length was 254 mm, internal volume was 2L, pressure transmitting medium was glycol (Houghton-Safe 620 TY, Houghton, Toronto, Ontario)/distilled water solution 50/50-v/v. Two samples were pressurised at one time. The pressures applied were 200, 300, and 400 MPa with settings of 20 °C for 10 min for the thermostatic bath. The pressure come-up time was 5 MPa/s, and the pressure release time was 10 s. Control and pressurised samples were stored in chilled conditions (+3 °C) in the dark and analysed at 0, 7, and 13 d of storage at 3 °C.

2.2. Microbial analysis

Samples were analysed for Total Aerobic Count and psychrotrophic bacteria, at 0d and after 13d. Samples were taken aseptically (5 g), sampling the skin and the underlying dorsal muscle portion, from the anterior-dorsal part of each fish, then stomached for 2 min in 0.1% sterile peptone water. Ten fold dilutions of the samples were made using 0.1% sterile peptone water and 0.1 ml aliquots of the appropriate dilutions were plated on Plate Count Agar (Merck, Danstadt, Germany). Plates were incubated at 30 °C for 72 h, for aerobic total count, and 4 °C for 10 days for psychrotrophic bacteria.

2.3. Water holding capacity

The ability of muscle to retain water was expressed as water holding capacity and determined according to Skipnes et al. (2007).

2.4. Stress relaxation test

Stress relaxation tests were performed with a TA.XT2i SMS Stable Microsystems Texture Analyzer. (Stable Microsystems Ltd., Surrey, England) using a 5 kg load cell. Samples were compressed by 5% with a 10 mm cylinder probe at a crosshead speed of 10 mm/s. The compression was kept constant for 100 s, allowing the stress to reach equilibrium. Measurements were taken from between the dorsal fin and the lateral line, parallel to the contact surface of the probe, taking three measures from each fish (Fig. 1). For each storage time (0, 7, 13 days), 5 fish for each treatment condition were analysed (control, 200, 300, 400), making a total of 20 fish per time of analysis. Texture Expert Exceed Software was used to acquire the data output.

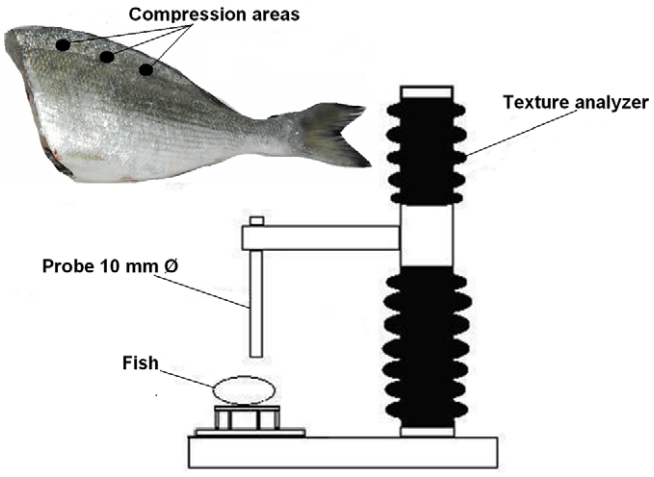

Fig. 1. Diagram showing the experimental setup and compression areas measured in each fish.