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Original research article

Effect of storage temperature and osmotic pre-treatment with alternative solutes on the shelf-life of gilthead seabream (*Sparus aurata*) fillets

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ABSTRACT

The objective of the study was the kinetic modelling of the shelf-life of osmotically pre-treated fish during refrigerated and super-chilled storage. Fresh gilthead seabream (*Sparus aurata*) fillets were treated for 0–360 min at 15 °C in osmotic solutions 50:5 high dextrose equivalent maltodextrin:NaCl/100 g (HDM), 40:10:5 HDM:trehalose:NaCl/100 g (HDM + treh) and 40:10:5 HDM:glucosamine:NaCl/100 g (HDM + gluc). Water loss, solid gain, salt content and water activity were monitored throughout treatment. Slices untreated and osmotically pre-treated for 45 min were aerobically packed and stored isothermally at 15, 10, 5, 2.5, 0, −1 and −3 °C. Quality assessment was based on microbial growth (total viable count, *Pseudomonas* spp., *Brochothrix thermosphacta*, *Enterobacteriaceae* spp., H_2S-producing bacteria, lactic acid bacteria, yeasts and moulds), total volatile nitrogen (TVB-N), lipid oxidation (TBARs) and sensory scoring. Quality indices were kinetically modelled and temperature dependence of quality loss rates was modelled by Ratkowsky equation.

Osmotic pre-treatment led to significant shelf-life extension of fillets, in terms of microbial growth, chemical changes and organoleptic deterioration. The pre-treatment with the alternative solutes led to depression of the freezing point (−1.8, −2.6, −3.2 and −3.5 °C for the untreated samples and the osmotically pre-treated with HDM, HDM + treh and HDM + gluc, respectively). TVB-N values were higher in untreated samples, followed by osmotically treated fillets, mainly at higher storage temperatures (i.e. 10 and 15 °C). Based on the mathematical models for sensory evaluation scoring, the shelf-life was 12, 19, 22 and 22 days at 0 °C for untreated and osmotically pre-treated with HDM, HDM + treh and HDM + gluc fish slices, respectively, while the respective values at −3 °C were 21, 35, 38 and 38 days. The alternative solutes had no significant effect on the quality and shelf-life of pre-treated fish fillet during storage at refrigerated conditions.

1. Introduction

Gilthead seabream (*Sparus aurata*) is one of the most cultured species in the Mediterranean area. Greece is a significant world marine fish producer, specifically for gilthead seabream and European sea bass, with the combined production capacity of about 110,000 tonnes in 2015 (FAO, 2016). Although usually sold as whole fish, filleted products have high commercial potential but suffer from short shelf-life. Spoilage of refrigerated fresh and minimally processed fish is attributed mainly to bacterial activity and it manifests itself as changes in the sensory characteristics (Gram & Huss, 1996).

Lightly preserved fish products are uncooked or mildly cooked products, with low level of preservatives (NaCl<6%, pH>5). These products are usually produced from fresh seafood and further processing involves one or a few additional steps (e.g. drying, salting, cold smoking etc) (Leroi et al., 2008). Recent studies investigate the potential hurdles that might contribute to ensuring the quality of lightly preserved fish products.

Osmotic dehydration (OD) is a technique used to reduce water activity (a_w) in foods in order to improve nutritional, sensorial and functional properties of food. It consists of an immersion of the product into a concentrated solution (i.e. sugar, salt, sucralose etc.). A driving force for water removal is set up because of a difference in

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osmotic pressure between the food and its surrounding solution (Collignan, Bohuon, Deumier, & Poligné, 2001; Raoult-Wack, 1994; Rastogi, Raghavarao, Niranjan, & Knorr, 2002). The potential of OD to extend the shelf-life of fish fillets by reducing the initial load and delaying microorganisms' growth has been reported by Tsironi and Taoukis (2012, 2014).

Super-chilling is a technology that consists of storing food just above the initial freezing temperature and helps to inhibit most autolytic and microbial reactions (Huss, 1995). During super-chilling the temperature of the foodstuff is lowered often 1–2 °C below the initial freezing point of the product. Several studies evaluate the effect of super-chilling storage (−0.5 °C to −4 °C) on seafood products, including the use of flake or slurry ice (Bahuaud et al., 2008; Rodríguez, Carriles, Cruz, & Aubourg, 2008) and storage at subzero temperatures (Chang, Chang, Shiau, & Pan, 1998; Duun & Rustad, 2008; Olafsdottir, Lauzon, Martinsdøttir, Oehlenschläger, & Kristbergsson, 2006; Sivertsvik, Rosnes, & Kleiberg, 2003). At super-chilling temperatures, microbial activity is limited, however chemical and physical changes may progress (Magnussen, Haugland, Hemmingsen, Johansen, & Nordtvedt, 2008). Recent studies investigate the potential use of cryoprotective agents during frozen storage of fish products (Fukuma, Yamane, Itoh, Tsukamasa, & Ando, 2012; Kaale and Eikevik, 2014).

The objective of the study was to evaluate and model the combined effect of osmotic dehydration with alternative solutes and storage at refrigerated (0 to 15 °C) and super-chilled (−3 to −1 °C) conditions storage on the shelf-life of chilled fillets of marine cultured gilthead seabream (*Sparus aurata*) fillets.

2. Materials and methods

2.1. Raw material

Marine cultured gilthead seabream (*Sparus aurata*) fillets (weight: 90 ± 10 g, capture zone: Aegean Sea, Greece) came from the same batch and were provided by a leading Greek aquaculture company. Fillets were transported directly to the laboratory in polystyrene boxes with appropriate quantity of flaked ice (0 °C) within 2–4 h. A polyethylene film was placed between layers of fillets, to avoid contact between skin and meat sides of the fillets.

Fillets were cut into rectangular slices ($3 \times 3 \times 1 cm^3$, 10±1 g) in a laminar flow hood. Osmotic solution was prepared by dissolving HDM, high dextrose equivalent (DE) maltodextrin (GLUCIDEX® 47 Syrope de Glucose Dehydrate, Roquette, France) and distilled water at a concentration of 50%. NaCl (5% ww) was added in the osmotic solution to increase the driving force of the process and attenuate the low level sweetness that could result from the HDM uptake during osmotic pre-treatment (Lerici, Pinnavaia, Rosa, & Bartolucci, 1985). Osmotic solutions with 40% HDM, 5% NaCl and 10% trehalose (TREHA™ British sugar, Felixstowe P/No. 1-2, Japan) or 10% glucosamine (Bioibérica, Barcelona, Spain) were also used (coded as HDM + treh and HDM + gluc, respectively). The concentrations of trehalose and glucosamine in the osmotic solutions were based on their solubility in water.

2.2. Osmotic pre-treatment

Sliced samples were osmotically treated at 15 °C for 0, 20, 40, 60, 90, 120, 180, 240, 300 and 360 min as described by Tsironi and Taoukis (2010). The solution to sample ratio was 5:1 (w/w) to avoid significant dilution of the medium by water removal, which would lead to local reduction of the osmotic driving force during process (Medina-Vivanco, Sobral, & Hubinger, 2002). Three replicate samples were removed and measured each time and the average values were taken.

Moisture content was determined by drying at 110 °C (WTB BINDER 7200, Type E53, Tuttlingen, Germany) for 24 h. Salt content of fish fillets was determined titrimetrically using silver nitrate solution by the Mohr method (AOAC, 1990). Sample water activity was determined using an a_w-meter (Rotronic AG, AM3+Aw VD, Bassersdorf, Switzerland). Water loss (WL) and solid gain (SG) were calculated according to Tsironi, Salapa, and Taoukis (2009).

2.3. Determination of freezing point of fish

The cooling curve is one of the most simple, accurate and widely used methods to measure the freezing point of foods. The wide application of this method is due to its accuracy and simplicity. In order to determine the freezing point of fish slices, raw slices were placed in a freezer (Whirlpool AFG 610 M-B chest freezer, Italy), at temperature of −30 °C. A type T thermocouple was inserted into the centre of the parallelepiped, temperature was constantly monitored and the cooling curve of fish was developed. The initial freezing point was determined from the cooling curve as described by Rahman (1995).

2.4. Shelf-life kinetic study

Samples were stored at controlled isothermal conditions of 15, 10, 5, 2.5, 0, −1 and −3 °C in high-precision (±0.2 °C) low-temperature incubators (Sanyo MIR 153, Sanyo Electric, Ora-Gun, Gunma, Japan) and shelf-life study was carried out. Temperature in the incubators was constantly monitored with electronic, programmable miniature dataloggers (COX TRACER®, Belmont, NC). Samples were taken in appropriate time intervals to allow for efficient kinetic analysis of quality deterioration.

2.4.1. Microbiological analysis

For microbiological enumeration, a representative sample (10 g) was transferred to a sterile stomacher bag with 90 mL sterilized Ringer solution (Merck, Darmstadt, Germany) and was homogenized for 60 s with a Stomacher (BagMixer® interscience, France). Samples (0.1 mL) of 10-fold serial dilutions of fish homogenates were spread on the surface of the appropriate media in Petri dishes for enumeration of different spoilage bacteria. Total aerobic viable count was enumerated on Plate Count Agar (PCA, Merck, Darmstadt, Germany) after incubation at 25 °C for 72 h. *Pseudomonas* spp. were enumerated on Cetrimide Agar (CFC, Merck, Darmstadt, Germany) after incubation at 25 °C for 48 h. *Brochothrix thermosphacta* was enumerated on STAA Agar (CM 881, Oxoid, Cambridge, UK) supplemented with SR 151 (Oxoid, Cambridge, UK) which was incubated at 25 °C for 48 h. Yeasts and moulds were enumerated on Rose Bengal Chloramphenicol Agar (RBC, Merck, Darmstadt, Germany) incubated for 168 h at 25 °C. For *Lactobacilli*, *Enterobacteriaceae* and H_2S-producing bacteria enumeration the pour-plate method was used. Lactic acid bacteria (LAB) were enumerated on De Man-Rogosa-Sharpe Agar (MRS, Merck, Darmstadt, Germany) followed by incubation at 25 °C for 96 h. For *Enterobacteriaceae* sp. enumeration Violet Red Bile Dextrose Agar (VRBD, Merck, Darmstadt, Germany) was used, which was incubated at 25 °C for 48 h. For the H_2S-producing bacteria Iron Agar was composed as described by Gram, Trolle, and Huss (1987) and incubated at 25 °C for 96 h.

Two replicates of at least three appropriate dilutions were enumerated. The microbial growth was modelled using the Baranyi Growth Model (Baranyi & Roberts, 1995). For curve fitting the program DMFit was used (available at http://www.combase.cc/index.php/en/). The kinetic parameter, rate (k) of the microbial growth, was estimated at all tested temperature conditions.

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