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Effect of chitosan treatments on quality parameters of fresh refrigerated swordfish (*Xiphias gladius*) steaks stored in air and under vacuum conditions

Maria Tsiligianni [a], Eikaterini Papavergou [b], Nikolaos Soultos [b], Taxiarhoula Magra [b], Ioannis N. Savvaidis [a,*]

[a] *Laboratory of Food Chemistry and Food Microbiology, Department of Chemistry, University of Ioannina, Ioannina 45110, Greece*
[b] *Laboratory of Food Technology, Department of Hygiene and Technology of Food of Animal Origin, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, GR 54124 Thessaloniki, Greece*

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ABSTRACT

The present study examined the effect of chitosan (1.0% w/v) in combination with packaging on the shelf-life of fresh swordfish steaks. Treatments included the following: A (untreated, control samples stored in air), A-CH (treated with chitosan 1.0% w/v, stored in air), VP (untreated, stored under vacuum packaging) and VP-CH (treated with chitosan, stored under vacuum packaging). VP-CH significantly affected Total Viable Counts (TVC), *Pseudomonas* spp., H_2S-producing bacteria (including *Shewanella putrefaciens*), lactic acid bacteria and *Enterobacteriaceae*. Production of TMA-N and TVB-N for A-CH, VP and VP-CH swordfish samples was significantly lower than for control (A) samples at day 8 of storage. Histamine formation for all treatments was low. A-CH and VP-CH resulted in significantly lower levels of putrescine, cadaverine and tyramine (day 8) as compared to A and VP swordfish samples. Results of this study indicate that the shelf-life of swordfish steaks can be extended using, either aerobic or vacuum packaging and in combination with chitosan, by approximately 4 (A-CH), 8 (VP) and 12 (VP-CH) days. Swordfish steaks treated with chitosan and stored under VP were sensorially acceptable up to 17 days. The presence of chitosan (A-CH and VP-CH) did not negatively influence the taste of cooked swordfish.

1. Introduction

Chitosan [poly-(-1/4)-2-amino-2-deoxy-D-glucopyranose] is a collective name for a group of partially and fully deacetylated chitin compounds (Kong et al., 2010). Due to its unique biological characteristics, including biodegradability and nontoxicity, many applications have been found alone or blended with other natural polymers (starch, gelatin, alginates) in the food, pharmaceutical, textile, agriculture, water treatment and cosmetic industries (Arvanitoyannis, 1999). Antimicrobial activity of chitosan has been demonstrated against many bacteria, fungi and yeasts possessing a high killing rate against Gram-positive and Gram-negative bacteria, but lower toxicity toward mammalian cells (Kong et al., 2010). Due to its antimicrobial activity, chitosan has attracted attention as a potential natural food preservative (Shahidi et al., 1999; Prashanth and Tharanathan, 2007). Development of natural preservatives with both antioxidant and antibacterial activities, that prolong the shelf-life of fish and prevent food-borne illness, is desirable.

Swordfish (*Xiphias gladius*) is one of the pelagic fish species belonging to the family of *Xiphidae*, mainly caught in the Atlantic, Pacific, Indian Ocean and temperate waters of the Mediterranean Sea. Swordfish is a fish of great consumption world-wide, due to its low fat content and high protein content. It is commonly sold for immediate consumption at retail stores as filleted fish (steaks), while ice storage has been widely used to prolong its shelf-life. Swordfish is a perishable food, due to bacteriological and enzymatic activity which takes place after death. Given that the shelf-life of refrigerated swordfish steaks is relatively short and that there is a growing tendency of consumers for fresh fish, the development of new preservation methods, resulting in shelf-life extension of fresh fish, is required.

To date there is limited information in the literature on fresh swordfish preservation. Little work has been reported on the application of packaging techniques applied either, singly (Lannelongue et al., 1982; Oberlender et al., 1983; Pantazi et al., 2008) or in combination with liquid smoking (Muratore and Licciardello, 2005), and with essential oils (EOs) such as oregano (Giatrakou et al., 2008) and thyme (Kykkidou et al., 2009).

Chitosan has been approved as a food additive in Japan since 1983. Increasing consumer demands for high-quality and microbiologically-safer foods, together with longer product shelf-life, are continuously forcing researchers and the industry to develop new food preservative strategies. Chitosan is mostly applied as a food additive or preservative, and as a component of packaging material, not only to retard microbial growth in food, but also to improve the quality and shelf-life of food (Giatrakou and Savvaidis, 2012). However, little work has been reported on the antimicrobial properties of chitosan against micro-organisms in fresh fish and seafood. To the best of our knowledge, the

* Corresponding author. Tel.: +30 26510 08343; fax: +30 26510 08795.
E-mail address: isavvaid@uoi.gr (I.N. Savvaidis).

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use of chitosan as a natural antimicrobial agent, applied either individually, or in combination, with aerobic or vacuum packaging, has not been studied to date, in fresh swordfish.

Thus, the objectives of the present study was to (1) study the effect of chitosan and packaging (air, vacuum) on the quality characteristics of fresh refrigerated swordfish steaks and (2) determine the shelf-life of the product stored in air or under vacuum, both in the absence or presence of chitosan using bacteriological, physicochemical, and sensory analyses at 4 °C.

2. Material and methods

2.1. Swordfish samples

Fresh whole swordfish (*X. gladius*) caught in the South Aegean Sea, with an approximate weight of 20–25 kg, were immediately beheaded, gutted, cleaned and put on ice. Fish were delivered to the laboratory on ice in insulated polystyrene boxes and in less than 24 h of landing. Immediately after delivery, whole fish was filleted manually; fillets were cut in steaks (ca. 150 g ± 15 g) subsequently kept under refrigeration in a cooling incubator (4 ± 0.5 °C) before the addition of chitosan (see below).

2.2. Preparation of chitosan solution

Chitosan of low molecular weight (MW; 340) in powder form (crab shells) was purchased from Aldrich Company (Athens, Greece). Moisture content was less than 10% and chitosan had a deacetylation degree of 75–85% (manufacturer's data). A stock solution of chitosan was prepared by dissolving 1.0 g in 100 ml of glacial acetic acid and stirred overnight at room temperature (final chitosan concentration = 1.0% w/v).

2.3. Application of chitosan to the swordfish samples

Chitosan was added to the samples using the following procedure: a swordfish steak sample (150 g ± 10 g) was transferred aseptically into an open low density polyethylene/polyamide/low density polyethylene (LDPE/PA/LDPE) pouch (VER PACK, Thessaloniki, Greece), 75 μm in thickness having an oxygen permeability of 52.2 $cm^3\ m^{-2}\ day^{-1}\ atm^{-1}$, at 75% relative humidity (RH), 23 °C, a carbon dioxide permeability of 191 $cm^3\ m^{-2}\ day^{-1}\ atm^{-1}$ at 0% RH, 23 °C and a water vapor permeability of 2.4 $g^{-2}\ day^{-1}$ at 100% RH, 23 °C. Chitosan with a final concentration equivalent to 0.045% w/w was added to the steak by spraying it directly onto the product. Chitosan was massaged onto the product so as to get an even distribution using gloved fingers to avoid cross-contamination of samples. Swordfish steak samples were separated into four lots: A, (untreated, control samples under aerobic storage in the absence of chitosan), VP, (untreated, samples under vacuum storage in the absence of chitosan), A-CH (treated, samples under aerobic storage, with added chitosan) and VP-CH (treated, samples under vacuum storage, with added chitosan).

2.4. Packaging of samples

Pouches were heat-sealed using a BOSS model N48 packaging machine (Bad Homburg, Germany) after air was drawn (vacuum storage) or not (aerobic storage). All samples (control, treated) were kept in a cooling incubator (Sanyo, Japan) for a period of 8 (A), 12 (A-CH) and 17 (VP, VP-CH) days under refrigeration (4 ± 0.5 °C). Three trials were carried out to test the efficacy of chitosan and packaging treatments on the quality of fresh swordfish fillets.

2.5. Microbiological analyses

Swordfish steak samples were cut aseptically into slices in a sterile container. Twenty five grams of swordfish steak slices were transferred into a stomacher bag and homogenized for 2 min (Lab blender 400, Seward Medical, London, U.K.), with 225 ml of peptone saline solution. From the resulting dilution, appropriate decimal dilutions were prepared, using the same diluent and plated in duplicate to enumerate the following microorganisms: a) Total Viable Counts (TVC), were enumerated by the pour–overlay method using Plate Count agar (PCA, Merck, Darmstadt, Germany) supplemented with 1% sodium chloride (NaCl) (Nickelson et al., 2001). Plates were incubated at 25 °C for 3 days. b) *Pseudomonas* spp. were enumerated using the surface-plate method on *Pseudomonas* agar base (CM 559, Oxoid, Basingstoke, UK) plus the selective agent *Pseudomonas* C–F–C supplement (SR 103 Oxoid). Plates were incubated at 25 °C for 2 days and an oxidase test was performed. c) H_2S-producing bacteria (including *Shewanella putrefaciens*) were enumerated by the pour–overlay method using iron agar (Agar Lyngby-IA; CM 867 Oxoid). Plates were incubated at 25 °C and black colonies formed by the production of H_2S were enumerated after 3 days. d) *Enterobacteriaceae* were enumerated by the pour–overlay method using Violet Red Bile Glucose (VRBG) agar (CM 485, Oxoid). Plates were incubated at 30 °C for 24 h. Purple colonies surrounded by the purple zone, were enumerated and recorded as *Enterobacteriaceae*. Finally lactic acid bacteria (LAB) were enumerated by the pour–overlay method using de Man Rogosa Sharpe (MRS) agar (CM361, Oxoid). Plates were incubated anaerobically at 30 °C for 3 days. LAB colonies were confirmed using the Gram (positive), catalase (negative) and oxidase (negative) tests. Average results of duplicate measurements, are presented as log colony forming units (CFU/g). Microbiological analyses of swordfish steaks took place on day 0, immediately after the filleting procedure of whole fish was completed, and every 2 days thereafter (except for day-17; VP, VP-CH treatments only) until unacceptable sensory characteristics developed as spoilage indicators.

2.6. Physicochemical analyses

Trimethylamine nitrogen (TMA-N) was determined using the method of AOAC (AOAC, 1990). Total volatile basic nitrogen (TVB-N) was determined using the method of Malle and Poumeyrol (1989). TMA-N and TVB-N contents were expressed as mg TMA-N/100 g or TVB-N/100 g fish muscle. Thiobarbituric acid (TBA) was determined according to the method of Botsoglou et al. (1994). TBA content was expressed as mg of malondialdehyde (MDA)/kg fish muscle. The pH value was recorded on each sampling day using a pH meter (Hanna Instruments, HI 9219, Woonsocket, RI, USA). Swordfish sample (10 g) was homogenized thoroughly with 90 ml of distilled water and the homogenate was used for pH determination. The extraction, separation and quantification of biogenic amines (BAs) were carried out using the procedure described by Veciana-Nogués et al. (1995).

2.7. Sensory analyses

Each swordfish sample (ca. 50 g) was cooked in a microwave oven at high power (700 W) for 10 min. A panel of seven judges experienced (laboratory-trained) in fresh fish evaluation was used for sensory evaluation. All panelists who evaluated the sensory attributes of cooked swordfish had previously participated in training sessions to become familiar with the sensory characteristics of cooked swordfish. Panelists were asked to evaluate odor and taste of the cooked samples. Acceptability as a composite of odor, taste and appearance was estimated using a scale ranging from 0 to 9. The scale points were: excellent, 9; very good, 8; good, 7; acceptable, 6; poor (early off-odor, off-taste, development) < 6; a score of 6 was taken as the lower limit of