



Effect of active packaging atmosphere on the shelf life of chilled stored steaks of barracuda *Sphyraena jello*

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ABSTRACT

In the present study, effect of an active reduced oxygen packaging atmosphere on the shelf life of barracuda (*Sphyraena jello*) fish steaks stored at 0-2°C was analysed. Barracuda steaks of 2.5 cm were divided into two batches; the first batch served as control, which was aerobically packed (AP) inside a multilayer film pouch of ethylene-vinyl alcohol (EVOH) with high barrier properties and the second batch packed in EVOH pouch was attached with a commercial oxygen (O₂) scavenger (OS). OS reduced the amount of O₂ inside the package to less than 0.01% within 24 h. On 12th day of storage, there was a 2 log difference in the total mesophilic count of steaks in OS attached pouches, compared to control. The 2 mg malonaldehyde kg⁻¹ limit of thiobarbituric acid (TBA) value, which is an index of secondary lipid oxidation, was exceeded in control, on 12th day of chilled storage. Based on the sensory score, control was rejected on 12th day, while OS steaks were acceptable till 20th day. The results indicate that OS is efficient in enhancing the storage life of fish by limiting the oxygen present inside the package.

Keywords: Active packaging, Barracuda, Oxygen scavenger, Shelf life

Introduction

Fish, which is a rich source of ω -3 poly unsaturated fatty acids (PUFA) and cheap protein, is a highly sought after food item worldwide. In 2010, global per capita fish consumption increased to 18.9 kg from an average of 9.9 kg in the 1960s (FAO, 2014), due to rapid population growth, increasing affluence and urbanisation. But, this preferred food spoils fast mainly because of microbial attack and oxidation of lipid, especially during long term storage. Since the rate of fish spoilage is highly temperature reliant, storing at low temperature is a very common and widely practiced fish preservation method (Sivertsvik *et al.*, 2002). In general, fish stored at chilled temperature, has a shelf life of 1-2 weeks, depending on the species. There are several reports on illegal practices of treating fish with unapproved chemicals or using permitted chemicals in more than allowable quantity, to further enhance the shelf life of chilled stored fish. In the present era of knowledge explosion, consumers are becoming more and more aware about the health implications of eating food containing synthetic additives. Thus, there is a huge demand for preservative free, minimally processed, and safer food which has led to the revolutionary concept of active packaging.

Unlike traditional packages, which act only as inert physical barrier, new generation active packages are intended to interact with the food contained inside or the surrounding environment (Pereira de Abreu *et al.*, 2012). A package becomes active, when the food, packaging and the nearby environment interact to lengthen the storage life of food, improving the sensory quality and/or food security (Suppakul *et al.*, 2003). This innovative concept can enhance the functionality of packaging because of the incorporation of an active substance into the packing material (Appendini and Hotchkiss, 2002). Currently, important active packaging components, which are getting popular, are oxygen scavengers, antimicrobial agents, ethylene absorbers, moisture regulators and carbon dioxide emitters.

Oxygen scavenging is the most widely adopted active packaging concept. Oxygen (O₂) is apparently one of the major reasons for fish spoilage because presence of oxygen causes oxidation of fish lipid and creates conducive atmosphere for the growth of aerobic microorganisms. Hence, restricting the amount of oxygen available in the package may enhance the storage life of extremely perishable foods like fish (Stiles, 1991). Most of the recognised O₂ scavengers are in the form of small sachets,

which contain different iron based powders combined with an appropriate catalyst. These kinds of scavengers usually react with moisture present in the food to produce a reactive hydrated metallic reducing agent that scavenges O_2 within the food package and permanently converts it to a stable oxide (Ozdemir and Floros, 2004). There are many advantages in using such oxygen scavengers. It simplifies the process of reducing the concentration of gases inside the package and also minimises the costs of equipment involved in other reduced oxygen packaging methods (vacuum and modified atmosphere packaging). They are efficient in bringing down O_2 level to <0.01% (Alvarez, 2000), which is much lesser than the usual 0.3–3.0% residual O_2 concentration attainable by modified atmosphere packaging. In spite of all these, there are a very few attempts to enhance the shelf life of fish by storing it in an active packaging atmosphere created using oxygen scavengers. Considering the above facts, the present study was aimed to improve the shelf life of barracuda (*Sphyraena jello*) fish steaks stored at 0–2°C using oxygen scavengers.

Materials and methods

Packaging and storage of barracuda fish steaks

Good quality barracuda (*Sphyraena jello*) fish was purchased from Veraval landing centre in Gujarat (India) and carried to the fish processing laboratory, with ice in 1 : 1 ratio. After reaching the laboratory, within half an hour, the fish were washed thoroughly and after beheading, gutting and cleaning, cut into steaks of 2.5 cm length and 50±5 g weight. The washed fish steaks were further randomly made into two batches. The first batch was packed under air (AP) inside multilayered plastic film pouch, which was made of EVOH (ethylene-vinyl alcohol) with nylon and polyethylene. The pouch was of 15×20 cm size and the thickness of the film was around 140 µm with an oxygen transmission rate of 3.86 ml m⁻² 24 h⁻¹ at 1 atmospheric pressure. The second batch of steaks (OS) was packed in EVOH pouch and a commercial O_2 scavenger (ZPT 200 EC, Ageless®, Mitsubishi Gas Chemical Company, Japan) with an O_2 absorption ability of 200 ml was attached inside the pouch. One O_2 scavenger sachet was placed inside the EVOH pouch containing one fish steak of 50±5 g weight. Pouches of both the batches were then heat sealed and stored at chilled temperature (0–2°C). Three pouches from each batch were drawn at regular intervals and subjected to headspace gas composition, biochemical, microbiological and sensory quality analyses.

Head space gas composition analysis

The gas composition in the headspace of the pouch was determined on every sampling day, including the first four days continuously, using a Gas analyser (PBI

Dansensor CheckMate 9900, Denmark). A syringe needle piercing a rubber septum pasted on the EVOH pouch was used for drawing the gas in the headspace.

Fish quality analysis

pH was measured using a digital pH meter (Cyberscan 510, Eutech Instruments, Singapore) after homogenising the fish sample in distilled water (1:5 w/v). Total volatile base nitrogen (TVB-N) and trimethylamine nitrogen (TMA-N) in the fish steak were determined by micro diffusion method (Conway, 1962). Primary and secondary lipid oxidation products in the samples were analysed by peroxide value (PV) (AOCS, 1989) and thiobarbituric acid (TBA) value (Tarladgis *et al.*, 1960). Hydrolytic rancidity during chilled storage was evaluated by free fatty acid (FFA) value of barracuda fish steak (AOCS, 1989). Plate count agar (PCA) was used for enumerating total mesophilic (Townley and Lanier, 1981) and psychrotrophic (Hitching *et al.*, 1995) counts of barracuda steaks by spread plate method. The inoculated plates were incubated at 37°C for 48 h and at 7°C for 5 days for total mesophilic counts and total psychrotrophic counts, respectively. A 9 point hedonic scale was used for organoleptic evaluation of the barracuda steaks. The sensory evaluation team had 6 regular members. They assigned a score of 9 for the most liked sample and 1 to the least liked sample (Meilgaard *et al.*, 1999).

Statistical analysis

SPSS (Statistical Package for the Social Sciences Inc., Chicago, USA, Version 17) software was used for data analysis. Means were compared using one-way and two-way ANOVA. Duncan's Multiple Range Test (DMRT) was used for determining the significant difference between the treatments. For microbiological quality, the level of significance was set up at $p < 0.01$ and for all other attributes, it was $p < 0.05$.

Results and discussion

Changes in the headspace gas (O_2 and CO_2) composition

Headspace gas (O_2 and CO_2) composition has been used as a useful indicator of microbial growth (Hurme, 2003). In the current study, there was a significant ($p < 0.05$) difference in both O_2 and CO_2 concentration in the air and O_2 scavenger packages, over the entire storage period (Fig. 1). Level of oxygen decreased to less than 0.01% within 24 h, inside the package with O_2 scavenger. The self triggering scavenger sachet enfolds iron based reactive compounds and the scavenging activity is due to iron oxidation. Similarly, decrease in O_2 concentration was reported for rainbow trout fillets pre-treated with oregano essential oil and packed with O_2 scavenger (Mexis *et al.*, 2009), for catfish (*Pangasius sutchi*) steaks packed

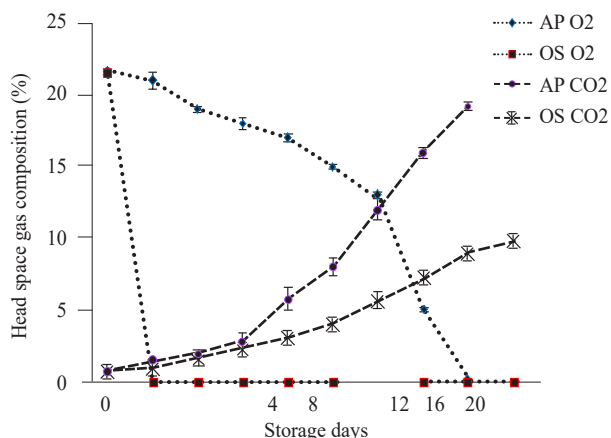


Fig. 1. Changes in the head space gas (O_2 and CO_2) composition* of barracuda steaks packed under different atmospheric conditions. *Mean \pm SD, n=3, p<0.05. AP: Air pack, OS: Pack with oxygen scavenger

with O_2 scavenger (Mohan *et al.*, 2008) and for gilthead seabream (*Sparus aurata*) packed with oxygen absorber (Goncalves *et al.*, 2004). O_2 content in aerobically packed barracuda steak samples also decreased slowly and reached 5% on 12th day. Level of CO_2 remained below 10% in O_2 scavenger packs, till the end of the storage period. Contrary to that, it increased steadily from 0.7% (day 0) to 19.2% (day 16) in air packs. The reduction of O_2 level and raise in CO_2 level in air packages is reportedly due to the proliferation of aerobic bacteria, which utilise the O_2 present in the package (Mohan *et al.*, 2010). CO_2 is produced in microbial metabolism and its accumulation in a package headspace is considered as a sign of microbial growth.

Changes in the quality of barracuda steak during storage

pH is used as an important factor to indicate the spoilage pattern of fish. In the current experiment, initial pH of barracuda steak was 6.1 (Fig. 2). In the early stages, pH decreased in aerobically packed sample and then increased steadily. Reduction of pH in the beginning is related to build up of lactic acid due to post-mortem breakdown of glycogen and could also be due to the CO_2 content in the packs. CO_2 dissolves in the tissue liquid leading to acidification and eventually carbonic acid is formed (Mohan *et al.*, 2008). The increase in pH in later period is presumably due to the growth of spoilage bacteria leading to the production of volatile basic amines (Pastoriza *et al.*, 1996). Barracuda steaks packed with and without O_2 scavenger had significant (p<0.05) difference in the pH values during storage. Samples packed under reduced oxygen atmosphere showed slower increase in pH indicating that the use of O_2 scavenger inhibited microbial growth and reduced the production of alkaline

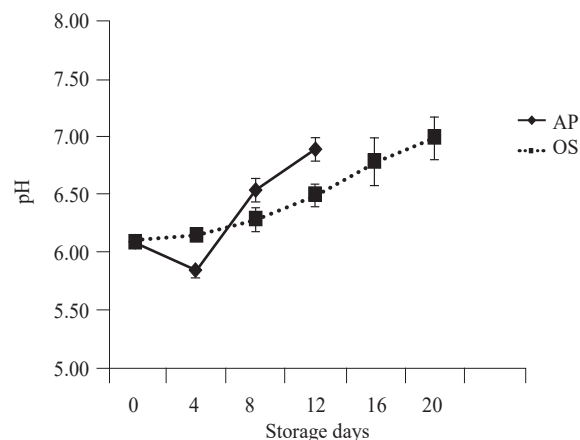


Fig. 2. Changes in the pH* of barracuda steaks in different packs during chilled storage. AP: Air pack, OS: Pack with oxygen scavenger. *Mean \pm SD, n=3, p<0.05

compounds.

Conventionally, the values of volatile base compounds, TVB-N and TMA-N, have been used as indicators to assess spoilage of fish products stored in ice. The initial TMA-N (Fig. 3) and TVB-N (Fig. 4) values of barracuda steak were 1.4 and 7.5 mg N_2 100 g^{-1} , indicating good quality of the raw material used for the experiment. On 12th day of chilled storage, the control sample exceeded the maximum acceptable limit of TMA-N, which is usually taken as 10-15 mg N_2 100 g^{-1} (Connell, 1995). TMA-N values of OS samples were below 10 mg N_2 100 g^{-1} till the end of storage period. The usual acceptable limit of TVB-N for fresh fish is 35-40 mg N_2 100 g^{-1} (Connell, 1990). The AP sample crossed this limit (36.4 mg N_2 100 g^{-1}) on 12th day of storage in ice, shortening its shelf life to only 12 days while O_2 scavenger significantly (p<0.05) delayed

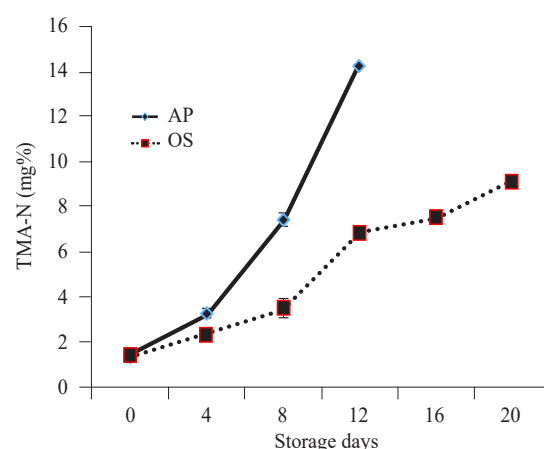


Fig. 3. Changes in the TMA-N value* of barracuda steaks in different packs during chilled storage. AP: Air pack, OS: Pack with oxygen scavenger. *Mean \pm SD, n=3, p<0.05

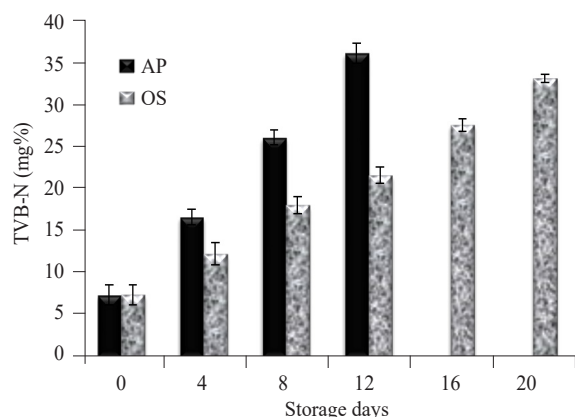


Fig. 4. Changes in the TVB-N value* of barracuda steaks during chilled storage. AP: Air pack, OS: Pack with oxygen scavenger. *Mean \pm SD, n=3, p<0.05

the TVB-N formation in OS sample. The O₂ scavenger decreased the O₂ concentration inside the package, which might have hindered the growth of aerobic bacteria and slowed down the formation of volatile basic amines (Ruiz-Capillas and Moral, 2005). Similarly, lower TMA-N and TVB-N values were observed for O₂ scavenger packed seer fish steaks (Mohan *et al.*, 2009).

Lipid hydrolysis leads to the formation of free fatty acids in fish. Though it does not cause any loss of nutritional value, its increase will lead to reduction in the acceptability of fish by consumers (Aubourg *et al.*, 1999). Therefore, FFA is quantified by many researchers to determine the sensory acceptability of food products (Barthet *et al.*, 2008). The initial FFA value of barracuda steak as % oleic acid was 1.7 \pm 0.15, which rose to 8.5 \pm 0.14 in AP (Fig. 5). The FFA value of aerobically packed sample was higher compared with samples packed under reduced oxygen atmosphere indicating the protective effect of O₂ scavenger. The final value of FFA on the day of sensory

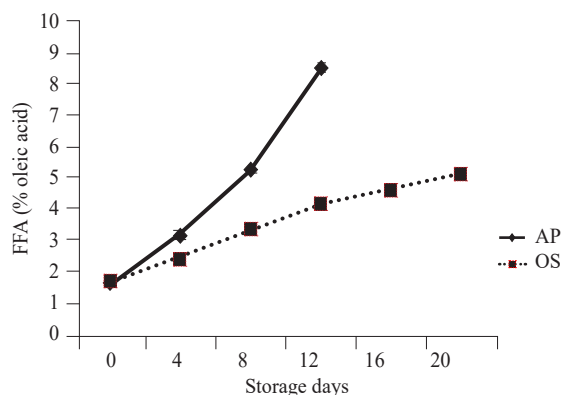


Fig. 5. Changes in the FFA value* of barracuda steaks during chilled storage. AP: Air pack, OS: Pack with oxygen scavenger. *Mean \pm SD, n=3, p<0.05

rejection in OS sample (20th day) was 5.1 \pm 0.13% oleic acid.

Peroxide value (PV) is an indicator of the primary lipid oxidation products in fish. PV of fresh barracuda steak was 0.94 meq O₂ kg⁻¹ sample (Fig. 6). In the initial part of storage, all the samples showed a continuous increase in PV, which varied with the packing atmosphere and PV decreased in the later part of storage. Unsaturated fat in the samples breaks down into primary products of lipid oxidation (the peroxides and hydroperoxides) causing an initial steady increase in PV. Further, hydroperoxide degradation, generating secondary lipid oxidation products like malonaldehyde, leads to decrease in the level of primary oxidation products (Boselli *et al.*, 2005). The control air sample showed a sharp increase in PV with storage time, whereas samples stored under reduced oxygen atmosphere had significantly (p<0.05) lower increase in the PV. Similar result was obtained by previous study for catfish stored with O₂ scavenger (Mohan *et al.*, 2008).

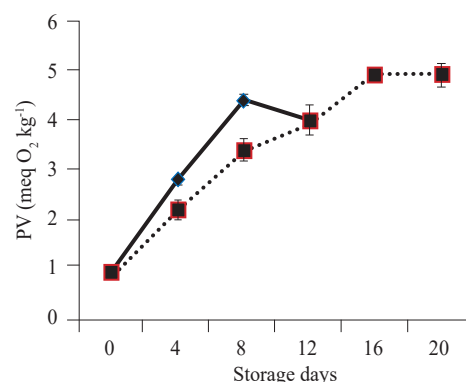


Fig. 6. Changes in the PV* of barracuda steaks in different packs during chilled storage. AP: Air pack, OS: Pack with oxygen scavenger. *Mean \pm SD, n=3, p<0.05

Thiobarbituric acid (TBA) value has been widely used to determine the secondary products of lipid oxidation. In the beginning, TBA value of barracuda steak was 0.32 mg malonaldehyde kg⁻¹ (Fig. 7). TBA value of barracuda steaks increased during chilled storage with a lower increase in sample packed with O₂ scavenger. Fish will usually have off-odour, when TBA value crosses 1-2 mg malonaldehyde kg⁻¹ of fish flesh and is generally considered as the limit (Fan *et al.*, 2009). The 2 mg malonaldehyde kg⁻¹ value was exceeded by control sample on 12th day of storage while samples packed with O₂ scavenger did not cross the limit till the end of storage. The higher increase in the TBA value of control could be due to exposure of sample to atmospheric oxygen entrapped in the air pack, which accelerates oxidation. In the same line, a reduction

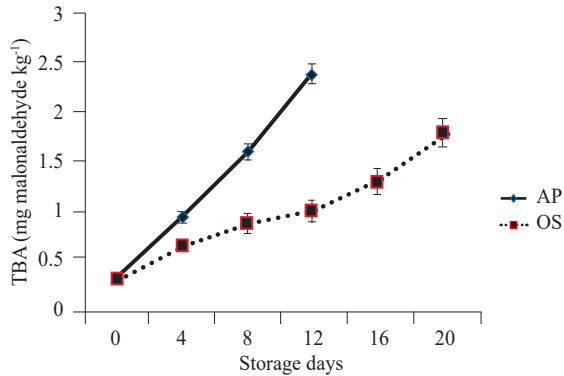


Fig. 7. Changes in the TBA value* of barracuda steaks in different packs during chilled storage. AP: Air pack, OS: Pack with oxygen scavenger. *Mean \pm SD, n=3, p<0.05

of malonaldehyde formation in sea bream packed with O₂ absorber compared with air packed sample was reported (Goncalves *et al.*, 2004).

In the beginning of the experiment, the total mesophilic (Fig. 8) and psychrotrophic counts (Fig. 9) of the barracuda steak were 3.3 and 2.5 log cfu g⁻¹, which increased over the period in fish steaks stored in different packaging environments. Initially, count of psychrotrophs recorded was less than mesophiles. As the storage of steaks progressed in ice, psychrotrophs dominated the bacterial flora due to rapid multiplication than mesophiles. At the end of the sensory life, psychrotrophic counts of AP and OS steaks were 8.2 and 7.6 log cfu g⁻¹. Ravishankar *et al.* (2008) also reported that psychrotrophic counts exceeded mesophilic counts in pearlspot samples stored at 0-2°C, regardless of packaging atmosphere. The count of total mesophilic and psychrotrophic bacteria increased significantly (p<0.01) in aerobically stored samples. The limit of 7 log cfu g⁻¹ of total mesophilic count was

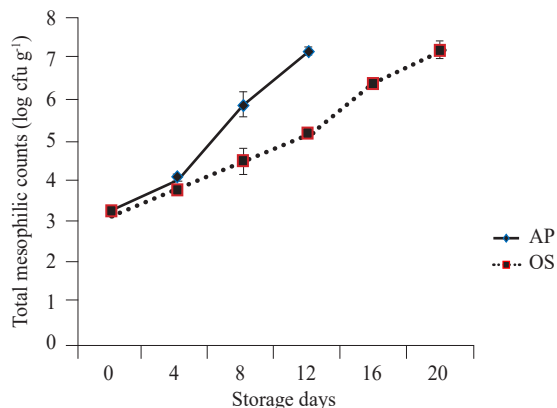


Fig. 8. Changes in the total mesophilic bacteria count* of barracuda steaks during chilled storage. AP: Air pack, OS: Pack with oxygen scavenger. *Mean \pm SD, n=3, p<0.01

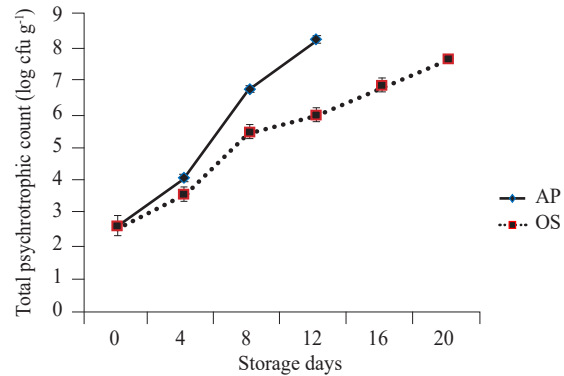


Fig. 9. Changes in the total psychrotrophic bacteria count* of barracuda steaks during chilled storage. AP: Air pack, OS: Pack with oxygen scavenger. *Mean \pm SD, n=3, p<0.01

surpassed by AP and OS samples on 12th and 20th days of storage, which coincided with the sensory rejection of the samples. Similarly, by the combined use of O₂ absorber and oregano oil, Mexis *et al.* (2009) observed an enhancement in the microbiological shelf life of rainbow trout fillets by 8 days.

Sensory analysis is one of the most important methods for judging the freshness and quality of fish. In the current work, appearance, colour, odour, taste, texture and flavour was assessed to evaluate the overall acceptability of the barracuda steak. Initially barracuda steak was very fresh with an overall acceptability score of 9 and had shiny appearance, seaweedy odour and firm and elastic texture, which altered with the storage period (Fig. 10). The results of the organoleptic evaluation (overall acceptability score) was well in agreement with the increase in the count of mesophilic bacteria and formation of volatile bases. Considering score of 5 as the lower limit of overall acceptability, the AP sample was

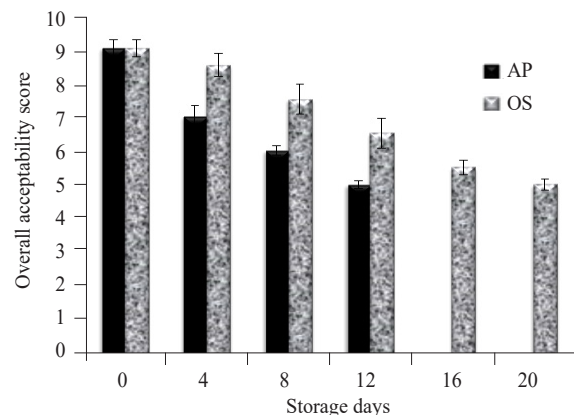


Fig. 10. Changes in the overall acceptability* of barracuda steaks in different packs during chilled storage. AP: Air pack, OS: Pack with oxygen scavenger. *Mean \pm SD, n=3, p<0.05

acceptable only for 12 days while the samples packed under reduced oxygen atmosphere had a sensory shelf life of 20 days. The results suggested that the active reduced packaging atmosphere created by O₂ scavenger could maintain the original organoleptic properties of fish, which are the first parameters assessed by consumers in grading fish and enhanced the shelf life of the barracuda steak stored at 0-2°C by 8 days.

Oxygen scavenger, the most popular active packaging technique can be used to reduce oxygen inside food packages to create a reduced oxygen atmosphere. The results of the present study clearly indicate that reduced oxygen concentration in the headspace of chilled stored barracuda fish steak pouches delayed lipid oxidation, volatile base formation and microbial spoilage action without affecting the sensory quality of fish leading to an extension in the storage life.

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