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Effect of Active Packaging on the Quality and Shelf Life of *Labeo rohita* during Chilled Storage

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ABSTRACT

The present study was undertaken to assess the efficacy of oxygen scavenger, the most widely used technique of active packaging, in extending the shelf life of fresh rohu (*Labeo rohita*) steaks during 28 days in chilled storage conditions (0-2 °C). Fresh rohu steaks were divided in two lots; the first lot was packed aerobically inside the EVOH (ethylene -vinyl alcohol) pouch, and the second lot was packed in EVOH pouch along with oxygen scavenger (OS). The oxygen scavenger effectively reduced the oxygen level inside the packs to <0.14% within 2 days of storage. During storage, a significant ($p<0.05$) reduction in rate of free fatty acid formation and lipid oxidation, indicated by thiobarbituric acid value (TBA) was observed for rohu fish steaks packed with oxygen scavenger in comparison to the air pack samples. The decreasing trend in proximate composition was also higher in air pack samples than those packed with oxygen scavengers. The limit of 7 log cfu $g^{-1}$of total plate count was crossed by control sample on 14th day of storage, while in oxygen scavenger packed sample, the total plate count reaches up to 6.78log cfu g^{-1}at the 28th day of storage. As per sensory evaluation, the air pack samples were rejected on 14th day, while oxygen scavenger steaks were acceptable till 28th day of storage.

Key words : *Active packaging, Labeo rohita, Oxygen scavenger, Shelf life*

Introduction

Fish is an indispensable source of food, as it is rich source of omega -3- polyunsaturated fatty acids (PUFA) and high-quality protein. Though fish is very beneficial, but it is highly perishable food and gets spoiled easily. Spoilage of fish muscle mainly occurs due to the changes caused by biological reactions like lipid oxidation, action of fish's own enzymes and by the metabolic activities of microorganisms (Ashie *et al*., 1996). All these processes result in the production of large amounts of free amino acids and volatile nitrogenous bases, therefore limiting the shelf life of fishes. Many preservation methods are employed to prevent the spoilage of fish. Chilling and refrigeration are the most preferred ones, as these techniques reduce the temperature of fish, increase the lag phase of bacteria and help in reducing spoilage (Mohan et al. 2019). Freshness of fish is rapidly lost even when stored in chilled environment due to the growth of aerobic microorganisms (Masniyom, 2011).In the present time, there are many reports of the employment of illegal ways of treating fish with unapproved harmful chemicals. But consumers now-a-days are much aware about health implications of eating food with synthetic food additives. (Remya *et al*., 2018). There is high demand of consumers for preservative free, high quality, fresh-like and safe food products (Ozdemir and Floros, 2004)and it has led to the development of active packaging techniques.

Active packaging has emerged as a new technique that performs 'desired role', by changing the conditions inside the package, instead of just pro-

viding inert physical barrier from external environment (Rooney, 1995). Thereby enhancing the shelf life, sensory property and maintaining the quality of food product. The important active packaging techniques that are effectively used for fishery products are the use of oxygen absorbers, carbondioxide emitters, moisture regulators, antioxidant films, antimicrobial agents etc. (Mohan *et al*., 2010).

The oxygen present inside the pack is mainly responsible for oxidation of fish lipids and provides favorable conditions for growth of aerobic bacteria. Thus, the most commonly adopted active packaging technique is the use of oxygen scavenger, that results into absorption of oxygen from headspace of package to value lower than 0.01% and maintains these conditions throughout the storage period to prevent the deteriorative changes in the fish muscle (Biji *et al.,* 2015). Different types of oxygen scavengers are available in the market based on principle of oxidation alone or in combination of one of the following components – iron powder, enzymes, ascorbic acid, photosensitive dye etc. (Cruz *et al.,* 2012). But majority of them are based on iron powder oxidation (Miltz and Perry, 2004). Oxygen scavengers have been extensively used to enhance the shelf life of catfish, seer fish etc. (Mohan *et al*., 2008, 2010).

Rohu (*Labeo rohita*), an Indian major carp, belongs to family Cyprinidae, has omnivorous feeding habit. This freshwater species is commercially important and has good market in India, owing to its fast growth rate and high nutritional value. Further, it undergoes degradative quality changes very rapidly, that limits its shelf life. Despite of all this, there is no report regarding the preservation of Rohu by using active packaging method. Therefore, the main objective of the present study was to evaluate the quality changes in *Labeo rohita* steaks packed in reduced oxygen atmosphere by the use of oxygen scavengers under chilled storage.

Materials and Methods

Preparation and storage of fish samples

The present experiment was conducted in fisheries laboratory of Department of Zoology, University of Jammu during November-December, 2020. Fresh *Labeo rohita* weighing about 600-650 gm with average length of 45-48 cm was purchased from local fish market in Trikuta Nagar, Jammu (India) and brought to the fishery laboratory within half an hour in iced conditionin insulated ice-boxes, with ice in 1:1 ratio. After reaching the laboratory, the fishes were immediately beheaded, gutted and washed. Then they were cut into steaks of 3.5cm length and 65±5gm weight. Fish steaks were divided into two lots.

One lot was packed inside pouches made of ethylene-vinyl alcohol film (EVOH) of size 15×20 cm, without oxygen scavenger, and it served as control air pack sample. The second lot of fish steaks was packed in EVOH pouch along with oxygen scavenger sachets named Ageless® ZPT 200 EC with an absorption capacity of 200 ml supplied by Oxy-mist absorbers, Gujarat, India. One fish steak weighing about 65gm was placed in a pouch together with one oxygen scavenger. All pouches were then heat sealed and stored under chilled conditions (0-2 °C). Sample pouches from both lots were checked at regular intervals of 7 days up to 28 days for proximate, biochemical, microbiological and sensory analysis.

Head space gas composition analysis

On every sampling day including the 2nd, 4th and 6th day, the gas composition (O_2 and CO_2) within the pouch was monitored by using the gas analyzer (PBI Dansensor, Checkmate 9900). The headspace gas sample was withdrawn through a syringe needle by puncturing the rubber septum pasted on EVOH pouch.

Fish quality analysis

Proximate composition analysis

The proximate composition of fish muscle was analyzed by following methods. Total crude protein and total lipid content was determined by using Lowry *et al.* (1951) and Folch *et al.* (1957) method respectively. For determining moisture and ash content the standard AOAC procedure was followed (AOAC, 2000a, 2000b). The moisture content was measured by drying the known amount of sample in hot air oven at 105±2 °C for 16 h and for measuring ash content the sample was heated in a muffle furnace at 550±2 °C for 4 h.

Physical analysis

Drip loss (%) was calculatedas $\frac{m_o - m_t}{m_o} \times 100$

where$m_o$was the initial weight (g) and m_t the weight

of fillet during sampling (g)

Chemical analysis

The pH was determined by homogenizing the fish muscle in distilled water in ratio 1:5(wt/vol) and the values were measured by using glass electrode attached to digital pH meter (LABPROPH 321). Oxidative stability in fish sample during the storage time was assessed by measuring the thiobarbituric acid value (TBA)according to the method described byTarlagdiset al. (1960)and calculated as mg malonaldehydekg^{-1}of fish sample. Free fatty acid (FFA) value of rohu steaks was determined by following AOCS (1989) procedure to evaluate lipid hydrolysis and it is expressed as percentage of oleic acid**.**

Microbiological analysis

The bacteriological study was done according to APHA (1984) method.10g fish muscle was mixed with 90 ml of sterile normal saline (0.9% NaCl) and then stomached for 2 min. Further, serial decimal dilutions were made with normal saline (0.9% NaCl) and then 0.5 ml of samples were transferred on plate count agar plates and inoculated plates were incubated at 37 ^{o}C for 2 days and at 7 ^{o}C for 5 days for enumeration of total plate count and psychrotrophic count respectively. For coliform count the violet red bile agar was used and 1ml sample of each dilution was pipetted by using pour plate method and plates were incubated for 24h at 37 ^{o}C. The results of microbial counts were expressed as logcolony forming units per gram of sample (log cfu g^{-1}).

Sensory analysis

The sensory quality evaluation of rohu fish steaks was conducted by a panel of six experienced members for uncooked fish samples on every sampling day using 9 point hedonic scale as described by Mohan *et al.* (2008) with modifications for rohu fish. The sensory attributes that were taken for evaluation include appearance, odor and texture. The fish samples were presented before the panelists and they were instructed to assign a score from 1 to 9 based on these attributes. A score below 5 was considered as rejected. The overall acceptability score was estimated by adding the scores of separate attributes and divided by total number of attributes.

Statistical analysis

All the experiments were performed in triplicates. The mean value and standard deviation were calculated for all the parameters, and all experimental data obtained were compared by applying analysis of variation (ANOVA) using the SPSS software version 24. Statistical significant differencebetween both samples for all the quality attributes were set up at $p< 0.05$.

Results and Discussion

Head space gas composition

The significant changes were found in headspace gascomposition ($O_2$and CO_2) concentration in both control and oxygen scavenger packages over the storage period. The oxygen content decreased, while carbondioxide increased in both packs (Fig. 1). A major difference occurred in the O_2 concentration inside the pouch having oxygen scavenger, within the first two days of storage as oxygen decreased from 21.1% to < 0.14%. At the 6thday, complete removal of oxygen occurred from the pack. And this level was maintained till the end of storage period, thus proving the effectiveness of oxygen scavenger composed of iron based reactive compounds that undergo oxidation to reduce the oxygen level. Similar observation on oxygen reduction was reported for Gilthead Seabram, in which oxygen level was reduced up to 0.1% after the 6th day of storage (Gonclaves *et al.*, 2004) and for rainbow trout fillet, where also the oxygen scavenger reduced the oxygen concentration up to less than 0.01% (Mexis *et al.* 2009) and these levels were maintained till the end of storage period. However, in the air pack samples this decrease was very slow, reaching to a value of 18.8% on 7th day. The decrease in oxygen content in air packed samples could be attributed to the growth of aerobic bacteria that consumed the oxygen present in the pack (Mohan *et al.*, 2019). Further the concentration of carbondioxide also rose in both pouches, but its level remained below 2.5% in Oxygen scavenger pack, while it increased up to 5% in control samples at the end of storage period. The accumulation of carbon dioxide in package headspace indicates the microbial growth as $CO_2$is produced in microbial metabolism. (Remya *et al.*, 2018).

Quality Assessment of Labeorohita

Proximate composition analysis

Proximate composition determines the nutritive value of fish muscle. The fresh fillets of *Labeo rohita*

used in the present study showed 17.60% crude protein, 1.78% crude lipid, 1.29% ash and 78.21% moisture content. These values were in range to the values reported by Sankar and Ramchandran (2001) for the same species. With respect to storage period, considerable differences were found in proximate composition of fish muscle in both control and Oxygen scavenger packed samples (Table 1). The percentage of crude protein decreased speedily from 17.60% to 13.47% in air pack samples, while the samples packed in oxygen free atmosphere had 15.65 % crude protein at last day of storage. During storage, there occur many changes in quality of fish that are related to the degradation and denaturation of muscle that ultimately leads to the loss in protein properties (Masniyom, 2011). The protein degradation in muscle can be attributed to the oxidation in myofibrillar protein. The sulfhydryl groups of myofibrils get oxidized in the presence of oxygen, resulting in the formation of disulfide bonds during cold storage. Further, these sulfhydryl groups are found abundant in the center of Ca^{2+} -ATPase of proteins and their oxidation strongly influence its activity. The Ca^{2+}-ATPase activity is also considered to be an important indicator of protein denaturation. Thus the presence of oxygen, causes oxidation of sulfhydryl groups, decreases the activity of Ca^{2+} -ATPase of actomyosinthat reflects the integrity of myofibrillar protein, thus ultimately indicating the degree of protein denaturation(Zhang *et al.*, 2019).

Apart from this, several microorganisms also develop on muscle, secreting variety of hydrolytic enzymes, mainly proteinases. Among such microbes, a strictly aerobic species like *Pseudomonas* sp. is particularly responsible for the deterioration of food proteins (Pantazi *et al*., 2008). Therefore, the probable reason behind the high amount of protein in oxygen scavenger packed muscle as compared to air packed ones is that the absence of oxygen in pack containing oxygen scavenger might have provided

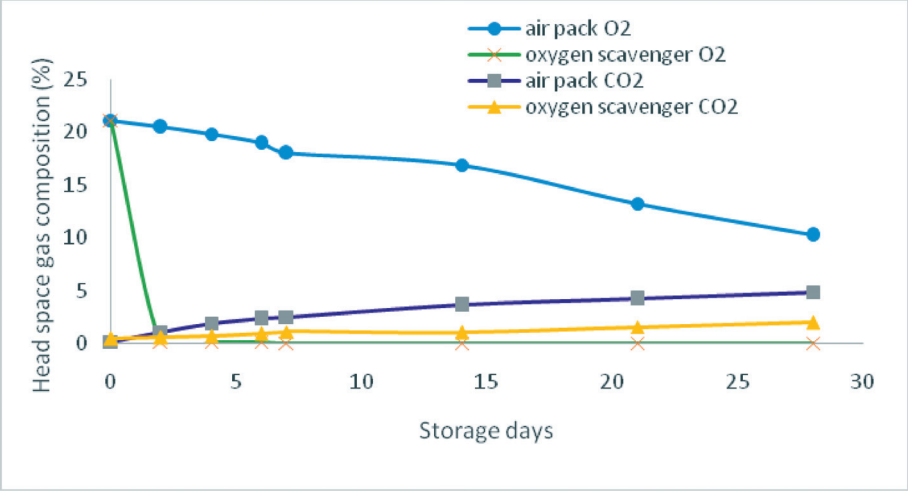

Fig. 1. Changes in gas composition of *Labeorohita* steaks during chilled storage in air and O_2scavenger pack. Mean ± SD, n=3, $p<0.05$

Table 1. Changes in proximate composition of *Labeo rohita* steaks during the chilled storage in air and O_2 scavenger packs.

		Storage days				
Attributes	Treatments	0	7	14	21	28
Protein (%)	Control	17.60± 0.72^{a}	16.85±0.64^{b}	15.53±0.55^{c}	13.76±0.58^{d}	12.89±0.62^{e}
	OS Pack	17.60±0.72^{a}	17.25±0.45^{a}	16.84±0.41^{b}	16.27±0.32^{c}	15.75±0.35^{d}
Lipid (%)	Control	2.34±0.18^{a}	1.96±0.17^{b}	1.45±0.15^{c}	1.13±0.13^{d}	0.85±0.12^{e}
	OS Pack	2.34±0.18^{a}	2.13±0.11^{a}	1.88±0.14^{b}	1.67±0.12^{b}	1.22±0.15^{c}
Moisture (%)	Control	78.21±1.26^{a}	75.92±1.32^{b}	73.87±1.37^{c}	71.23±1.24^{d}	67.54±1.26^{e}
	OS Pack	78.21±1.26^{a}	77.53±1.23^{a}	76.71±1.12^{b}	75.69±1.31^{b}	74.33±1.15^{c}
Ash (%)	Control	1.29±0.06^{a}	1.14±0.04^{b}	1.02±0.03^{c}	0.83±0.05^{d}	0.69±0.06^{e}
	OS Pack	1.29±0.06^{a}	1.22±0.02^{a}	1.17±0.04^{b}	1.08±0.03^{c}	0.94±0.02^{c}

$^{a\text{-}e}$Mean ± SD with different superscripts in a column differs significantly ($p<0.05$)

the protective effect against oxidation of proteins of fish muscle and it may also halted the growth of various aerobic microorganisms during chilled storage, thus resulting into the stability of crude protein content in fish muscle of these packs in comparison to the air pack sample. Some authors also observed similar results regarding protein oxidation. Zhang *et al*. (2019) observed the inhibitory effect of Modified atmosphere packaging and vacuum packaging on the protein oxidation of grouper fillets. Demirhan and Candogan (2017) also reported that the incorporation of oxygen scavenger in modified atmosphere packaging system (MAP) resulted delay in protein oxidation as well as retardation in growth of total aerobic bacteria and *Pseudomonas* sp. of chicken.

The percentage of lipid was also higher in oxygen scavenger packed sample as it was 1.56% on 14thday in comparison to air pack samples having 1.32% on same day of storage as fish lipids are rich in long chain polyunsaturated fatty acids that are highly susceptible to oxidation during processing and storage (Masniyom 2011). In the present study, in case of oxygen scavenger packs, lipid oxidation and lipolysis of fish muscle was prevented in oxygen free environment that might have maintained the level of lipids in these samples, while in air pack samples, the lipid deterioration occurred at faster rate, leading to the loss of lipids.Mahboob *et al.* (2004) also reported that decrease in lipid content may be linked with oxidation of polyunsaturated fatty acids found in fish tissue to other products like aldehydes, ketones and peroxides.

Likewise, moisture content reduced up to74.33% and 69.54% on 28th day in oxygen scavenger packed samples and control air pack samples respectively. The decline in moisture content of air packed samples might be associated with the loss of water holding capacity of myofibrillar proteins due to their oxidation in presence of oxygen,as the postmortem structural changes like denaturation of myosin and myofilament degradation is related to low water holding capacity (Dawson *et al.* 2018). Further, myofibrils constitute 80% of volume of living muscle fibres, and most of the water in muscle cell is held in myofibrils in spaces between thick and thin filaments (Den Hertog-Meischke *et al*., 1997). In oxygen scavenger packed sample, the oxidation of myofibrils occurred at slower rate that maintained the moisture content of fish muscle for longer period. Similar reduction in ash content was also observed in both air pack and oxygen scavenger packed sample.The reduction in ash content was associated with the drip loss during thawing process. (Gandotra *et al.*, 2016). Gandotra *et al*. (2015) also observed similar decreasing trend in proximate composition for vacuum packaged *Labeo rohita* stored under frozen conditions.

Chemical analysis

Changes in Thiobarbituric acid (TBA) value

TBA value is mainly used to assess the extent of secondary lipid oxidation by measuring the malonaldehyde content. The fish muscle develops unpleasant taste and odor when TBA content rises above 1-2 mg malonaldehyde kg^{-1} and therefore this is regarded as limit. (Connell, 1995). In the current study, TBA content showed a regular increase in all

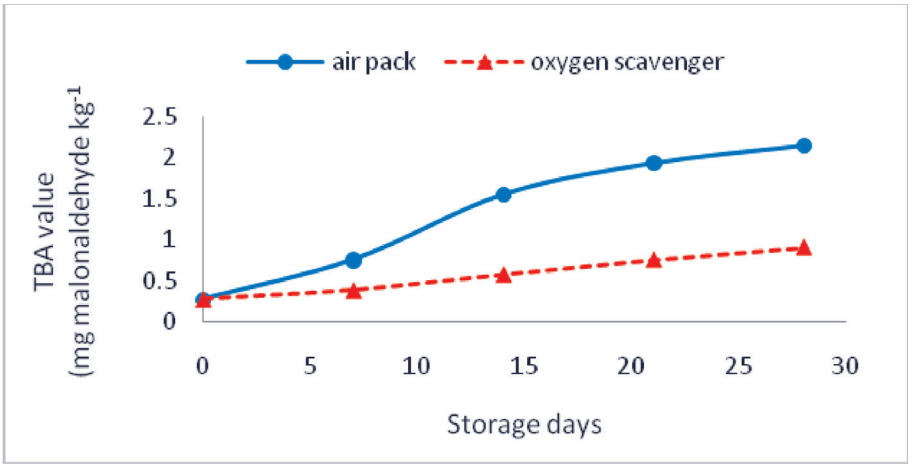

Fig. 2. Changes in thiobarbituric acid (TBA) value of *Labeo rohita* steaks during the chilled storage in air and O_2 scavenger packs. Mean ± SD, n=3, p<0.05

the samples during storage time (Fig. 2). A statistically significant difference was found between both the samples after the 7th day of storage. The initial value of TBA in *Labeorohita* was 0.28 mg malonaldehyde kg^{-1} and it exceeded 1mg malonaldehyde kg^{-1} for control fish samples on the 14^{th}day of storage, while a lower value of 0.91 mg malonaldehyde kg^{-1} in oxygen scavenger packed samples was observed at end of the storage period. This is mainly due to the ability of oxygen scavenger to maintain the level of oxygen<0.14% within the pack throughout the storage time, which is responsible for reducing the rate of oxidation of hydroperoxides, as they are initial product formed after oxidation of polyunsaturated fatty acid, that oxidize rapidly in aerobic conditions to form malondialdehyde (Erkan, 2012). Similar results with oxygen scavenger were reported by many authors. Remya *et al.* (2016) reported that cobia fish steaks packed with oxygen scavenger have effectively reduced TBA value, during the storage at 2 °C. Mohan *et al.* (2008) also observed reduction in malonaldehyde formation in catfish steaks packed with oxygen scavenger.

Changes in Free fatty acid

The free fatty acid formation in fish muscle is mainly due to chemical and enzyme mediated hydrolysis, and the degree of lipid hydrolysis can be evaluated by measuring the amount of free fatty acid (Biji *et al.*, 2016). FFA content in *Labeo rohita* fillets in O_2 scavenger packs and control samples are presented in Fig. 3. At the beginning of storage, the value of FFA was 0.35% oleic acid g^{-1} that gradually increased in all the samples. Its rate of production was faster in control samples, as it increased to 3.8% oleic acid g^{-1} fat on 14th day of storage period. However, slow increase was found in oxygen scavenger packed samples, with a value of 3.71% oleic acid g^{-1} at the end of storage period. Similar results for FFA content were also observed for oxygen scavenger packed barracuda steaks (Remya *et al.*, 2018). Solanki *et al.* (2019) also reported reduced rate in production of free fatty acid in dried sardines packed with oxygen scavenger. The results obtained indicate that reduction of oxygen might have delayed the hydrolysis of phospholipids and triglycerides by limiting the growth of aerobic bacteria and also by inactivating the lipolytic enzymes that are responsible for deterioration of fish lipids. (Mohan *et al.*, 2019).

Changes in pH

Variation in the values of pH in rohu fillets packed with and without oxygen scavenger during chilled storage are depicted in Fig 4. The initial pH of fish sample was 6.51. In control air pack samples the pH initially decreased and later there was a continuous sharp increase, taking it to a value of 6.82 on their sensory rejection on 14^{th} day, whereas pH values of oxygen scavenger packed samples were fairly stable, with values of 6.61, 6.68 and 6.75 at 14^{th}, 21^{th} and 28^{th} day respectively. The possible reason for the initial decrease in pH of air packed samples might be the presence of CO_2 that on coming in contact with tissue fluid, may have led to the produc-

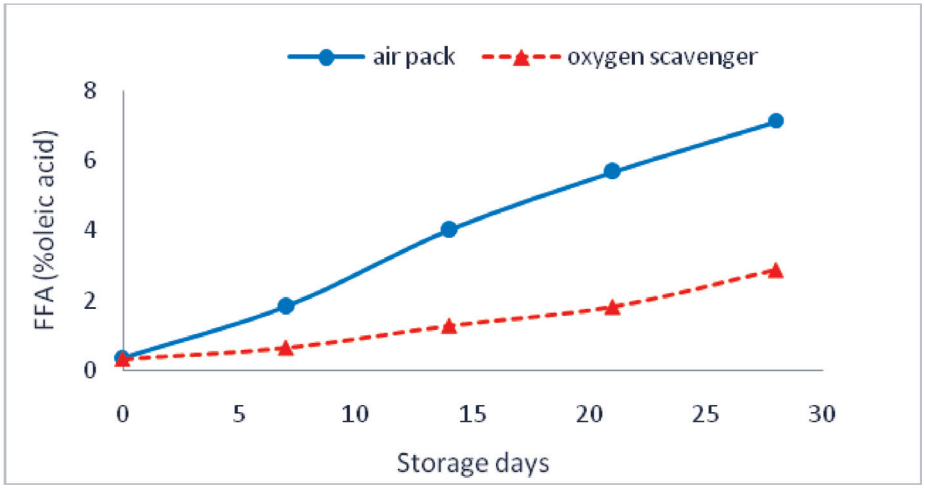

Fig. 3. Changes in FFA values of *Labeorohita*steaks during chilled storage in air and O_2 scavenger pack. Mean ± SD, n=3, p<0.05

tion of carbonic acid, thus creating an acidic environment, ultimately lowering the pH. The gradual rise in pH of fish fillets over the storage period is attributed to the formation of compounds such as ammonia and trimethylamine, produced as a result of bacterial growth and action of endogenous enzymes (Karoui and Hassoun, 2017). But this decrease was slower in samples packed with oxygen scavenger, owing to the presence of lactic acid producing bacteria in reduced oxygen atmosphere (Mohan *et al.*, 2019).

Physical analysis

Changes in Drip Loss

Drip loss represents the physical changes that occurred inside the pack due to loss of water soluble components from the fish muscle, which affect the consumers appeal. Its level increased all along the storage period in both the samples. Results showed higher drip loss for air pack samples with a value of 11.2% in comparison to oxygen scavenger packs having value of 4.3% at the end of storage period. (Fig. 5). Higher drip loss observed for air pack samples is probably because the initial fall of pH favors the exudation process, as it is generally recognized that there is a negative correlation between pH and driploss (Dalgaard *et al.*, 1993). Some authors like Mohan *et al.* (2019) reported higher drip loss for air packed Indian oil sardine since the muscle had lost the less tightly bound water due to the denaturation of myosin, present in myofibrils thatform muscle bundles in fish.

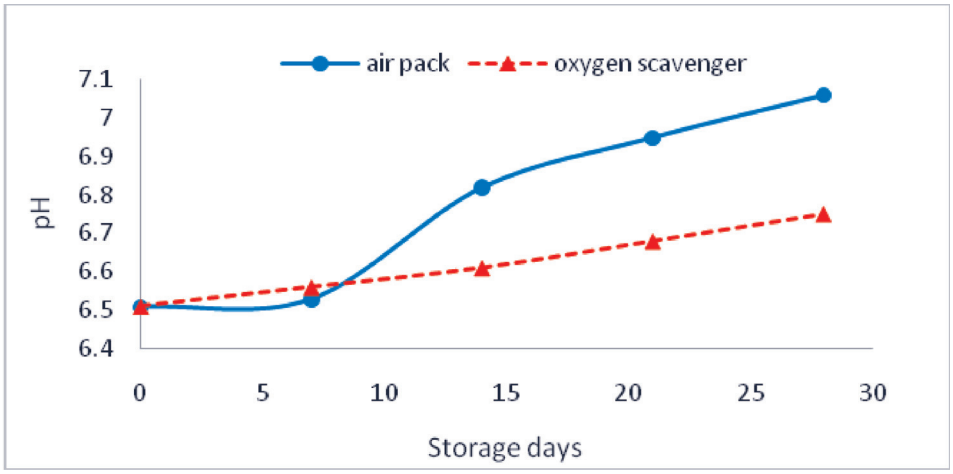

Fig. 4. Changes in pH values of *Labeo rohita*s teaks during chilled storage in air and O_2 scavenger pack. Mean ± SD, n=3, p<0.05

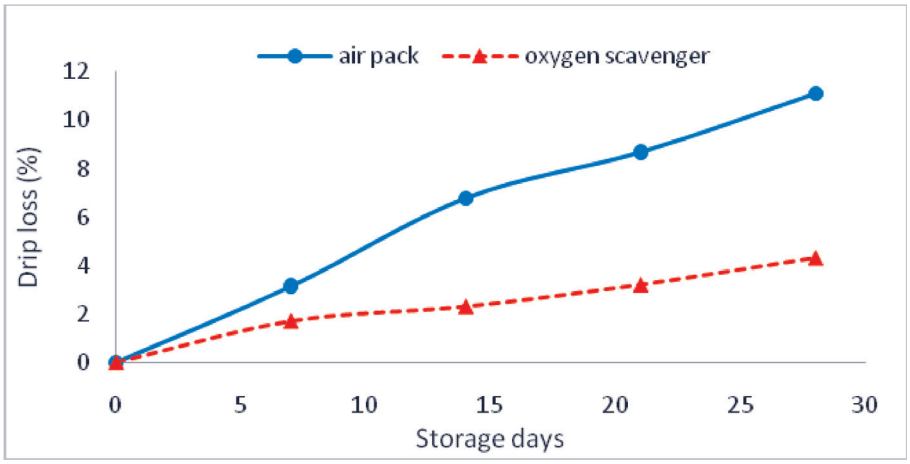

Fig. 5. Changes in drip loss (%) of *Labeo rohita* steaks during the chilled storage in air and O_2 scavenger packs Mean ± SD, n=3, p<0.05

Microbial analysis

Microbial growth is the major factor that is responsible for the deteriorative changes in freshness of fish such as off odors and flavors (Huss, 1988). Total plate count determines the microbial quality and spoilage of perishable food products. The Changes in Total plate count and psychrotrophic count insamples packed under different atmosphere with respect to storage time are given in Fig 6a,6b respectively. The TPC (aerobic mesophilic flora) and psychrotrophic count of fresh fish sample were 3.54 and 2.98 log cfu g^{-1} respectively, demonstrating the good quality of fish fillets. The fish samples packed with oxygen scavenger showed an initial lag phase for TPC up to7 days. Similarly, a lag phase of 5 dayswas reported for cobia fish steaks wrapped with antimicrobial film and packed with oxygen scavenger sachet (Remya *et al.*, 2016). A relatively long lag phase apparently represents bacteria adapting to altered atmosphere before multiplication (Mohan *et al.*, 2019). The extension in the lag phase and generation time, that was observed in this study, showed the inhibitory effect of O_2 scavenger on microbial growth. Afterthe lag phase of 7 days, TPC showed an increasing trendand reached to a final value of 6.78log cfu g^{-1} at end of storage period which is below the limit proposed byICMSF (1986). While the control samples attained a total plate count of 7.05 log cfu g^{-1} on day 14, indicating a microbiological shelf life of about 14 days for control samples. On similar account, a significant difference was observed in psychrotrophic count for samples packed under different atmosphere. At the start of experiment, their number was less than TPC count but as storage time progressed, psychrotrophic bacteria multiplied rapidly than aerobic mesophilic

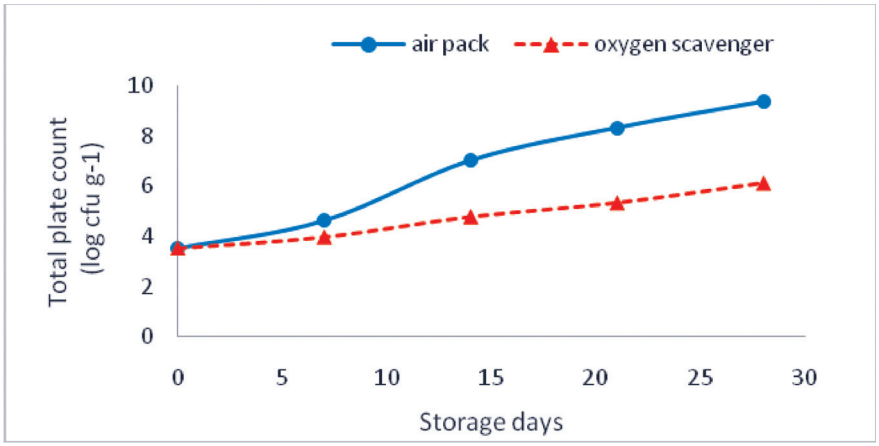

Fig. 6. Changes in total plate count (6a), Psychryotrophic count (6b) of *Labeo rohita*s teaks during the chilled storage in air and O_2 scavenger packs Mean ± SD, n=3, $p<0.05$

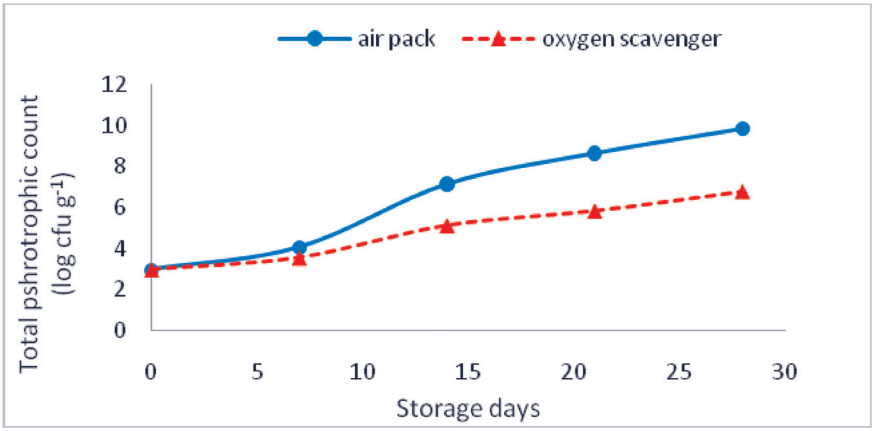

Fig. 7. Changes in overall acceptability score of *Labeo rohita* steaks during the chilled storage in air and O_2 scavenger packs Mean ± SD, n=3, $p<0.05$

bacteria, thus dominatingthe bacterial flora with a count of7.01 and9.54 log cfu g^{-1}in oxygen absorber pack and control air pack respectively, at the last day of storage. These results of microbiological shelf life in all fish samples coincided with sensory rejection. Other researchers also inferred similar microbiological results by using oxygen scavenger for different fish species such as Mohan *et al.* 2008 observed shelflife extension of 20 days for catfish steaks by use of oxygen scavenger.

The coliform count determines the sanitary conditions during the food processing (Houchier *et al.* 2013). The initial value for coliform count was 1.71 log cfu g^{-1} and it increased over the storage period in both the groups However, there was no considerable difference between the Coliform count of differently packed samples. This might be due to the facultative anaerobic nature of these bacteria.The results obtained from the current study have shown the extension in microbiological shelf life of *Labeo rohita* by the use of oxygen scavenger and is attributed to the fact that the spoilage action in aerobically packed fish sample might be due to presence of gram negative psychrotrophic non fermenting rods and among them, *Pseudomonas* spp. and *Shewanella* spp have been reported as dominant species that tend to spoil the chilled fish (Gram and Huss, 1996). But in case of oxygen scavenger packed fish sample, the oxygen reduced atmosphere created by the presence of oxygen scavenger might have negatively affected the growth of aerobic pseudomonads. These observations are in agreement with those of Mexis *et al.*, 2009 who reported the reduction of Pseudomonads by 1.9 log cfu g^{-1} for oxygen scavenger packs in comparison to control air packs for rainbow trout fillets at the 5^{th} day of storage period.In the similar way, Remya *et al.* (2016) have also observed the significant difference ($P< 0.01$) in *Pseudomonas* spp. Count of fish samples packed with oxygen scavenger.

Sensory analysis

In the initial phase, rohu fillets were very fresh having the overall acceptability score of 9 with a shiny appearance, slightly muddy odor and firm texture. The results of sensory evaluation of *Labeo rohita* showed differences in overall acceptability score between air pack and oxygen scavenger packed samples and are presented in the Fig 7. The fish fillets packed with oxygen scavenger obtained higher sensory scores throughout the storage period than the control ones. The fish samples were considered to be in acceptable state until they reached the score of 5, beyond which they were not recommended for human consumption. On this basis, the limit of acceptability of raw fish fillets was 14 days for control samples with an overall score of 4.6 on the same day and 28 days for Oxygen scavenger packed samples having a score of 5.2. These observations were in agreement with microbiological results and chemical analysis.

Conclusion

The present work proved the ability of oxygen scavenger (Ageless®) to limit the microbial spoilage, delaying the lipid oxidation process without affecting its sensory quality by complete removal of oxygen from the package after 6^{th} day, thus enhancing the shelf life of fresh *Labeo rohita* up to 28 days, whereas aerobically packed samples had shelf life of only 14 days. Further, the oxygen scavenger is economically viable as it reduces the cost of equipment that is required in other packaging techniques (Vacuum packaging and modified atmosphere packaging). Thus, the packaging with Oxygen scavenger is advantageous to maintain the freshness of fish for longer period.

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References

AOAC. 2000a. Methods 920.36. *Dry matter on oven drying. In: Official methods of analysis*, chapter 4. Association of Official Analytical Chemists International, VolI. 17th edn., Gaithersburg.1–2.

AOAC. 2000b. Methods 938.08 *Ash of Seafood. In: Official methods of Analysis,* chapter 39. Association of Official Analytical Chemists International. VolII. 17th edn.Gaithersburg. 8–9.

AOCS. 1989. *Official Methods and Recommended Practices of American Oil Chemists Society*, 5th edn. A.O.C.S., Champaign.

APHA 1984. *Compendium of Method of Microbiological Ex-*

amination of Foods. 2nd Edn. American Public Health Association, Washington DC.

Ashie, I.N.A., Smith, J.P and Simpson, B.K. 1996. Spoilage and Shelf-life Extension of Fresh Fish and Shell Fish. *Critical Reviews in Food Science and Nutrition*. 36 (1&2) : 87-12.

Biji, K.B., Ravishankar, C.N., Mohan, C.O. and Srinivasa Gopal, T.K. 2015. Smart Packaging systems for food applications: a review. *Journal of Food Science and Technology*. 52(10) : 6125-6135.

Connell, J.J. 1995. Intrinsic quality. In: *Control of Fish Quality*. Fishing news books. Wiley, London, pp 5–36.

Cruz, R.S., Camilloto, G.P. and Pires, A.C.D.S. 2012. Oxygen Scavengers: an approach on Food Preservation. *In Tech.* 2 : 21-42.

Dalgaard, P., Gram, L. and Huss, H.H. 1993. Spoilage and shelf- life of cod fillets packed in vacuum or modified atmospheres. *International Journal of Food Microbiology*. 19 : 283-294.

Dawson, P., Al- Jeddawi, W. and Remington, N. 2018. Effect of freezing on the shelf life of salmon. *International Journal of Food Science.* 1-12.

Demirhan, B. and Candogan, K. 2017. Active packaging of chicken meats with modified atmosphere including oxygen scavengers. *Poultary Science*. 96 (5) : 1394–1401.

Den Hertog-Meischke, M.J.A., Van, L. aack R.J.L.M. and Smudlers, F.J.M. 1997. The water-holding capacity of fresh meat. *Veterinary Quarterly.* 19(4) : 175-81.

Erkan, N. 2012. The effect of garlic oil on the preservation of vacuum packaged hot smoked rainbow trout (*Oncorhynchus mykiss). Food Bioprocess Technology*. 5: 1246-1254

Folch, J., Less, M. and Sloane, G.W.S. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*. 226 : 497-509.

Gandotra R., Raj S., Sharma M. and Kumari R. 2016. Studies on the effect of vacuum packaging on biochemical and microbial quality of frozen stored muscle of common carp, *Cyprinus carp*. *International Journal of Recent Scientific Research.* 7(10) : 13993-13998.

Gandotra, R., Koul, M., Gupta, S. and Gupta, V. 2015. Studies on the effect of vacuum packaging on some quality changes in *Labeorohita* during frozen storage period. *International Journal of Applied Biology and Pharmaceutical Technology*. 6(2) : 78-84.

Gonclaves, A., Mendes, R. and Nunes, M.L. 2004. Effect of Oxygen Absorber on the Shelf Life of Gilthead seabram (*Sparus aurata*). *Journal of Aquatic Food Product Technology*. 13(3): 49-59.

Gram, L. and Huss, H.H. 1996. Microbiological spoilage of fish and fish products. *Int. J. Food Microbiology*. 33 : 589–595.

Houicher, A., Kuley, E., Ozogul, F., Bendedxadouche, B. and Ozogul, F. 2013. Effects of natural extracts (*Mentha spicata* L. and *Artemisia campestris)* on shelf life and quality of vacuumpacked refrigerated sardine (*Sardina pilchardus*) fillets. *Journal of Food Protection.* 76 (10) : 1719-1725.

Huss, H.H. 1988. *Fresh fish, quality and quality changes*. FAO fisheries series, no. 29. FAO, Rome, p 132.

ICMSF 1986. Sampling plans for fish and shellfish. In: International commission on microbiological specifications for foods, microorganisms in foods. *Sampling for Microbiological Analysis: Principles and Scientific Applications,* 2nd edn. University of Toronto Press, Canada, vol 2, pp 181–196

Karoui, R. and Hassoun, A. 2017. Efficiency of rosemary and basil essential oils on the shelf life extension of atlantic mackerel (*Scomberscombrus)* Fillets stored at 2°C. *Journal of AOAC International*. 100 (2): 335-344.

Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J. 1951. Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry*. 193:265-275.

Mahboob, S., Liaquat, F., Liaquat, S., Hassan, M. and Rafique, M. 2004. Proximate composition of meat and dressing losses of wild and farmed *Labeo rohita* (Rohu). *Pakistan Journal of Zoology*. 36: 39-43.

Masmiyom, P. 2011. Deterioration and shelf –life extension of fish and fishery products by modified atmosphere packaging. *Songklanakarin Journal of Science and Technology*. 33(2): 181-192.

Mexis, S.F., Chouliara, E. and Kontominas, M.G. 2009. Combined effect of an oxygen absorber and oregano essential oil on shelf life extension of rainbow trout fillets stored at 4°C. *Food Microbiology*. 26 : 598-605.

Miltz, J.B. and Perry, M. 2004. Evaluation of the Performance of Iron-based Oxygen Scavengers, with comments on their Optimal Applications. *Packaging Technology and Science*. 18 : 21-27.

Mohan, C.O., Abin, J., Kishore, P., Panda, S.K. and Ravishankar, C.N. 2019. Effect of Vacuum and Active Packaging on the Biochemical and Microbial Quality of Indian Oil Sardine (*Sardinella longiceps*) during iced storage. *Journal of Packaging Technology and Research.*

Mohan, C.O., Ravishankar, C.N. and Srinivasa Gopal, T.K. 2008. Effect of O_2 Scavenger on the shelf-life of Catfish (*Pangasius sutchi*) Steaks during chilled storage. *Journal of the Science of Food and Agriculture*. 88 : 442-448.

Mohan, C.O., Ravishankar, C.N. and Srinivasa Gopal, T.K. 2010. Active Packaging of Fishery Products: A Review. *Fishery Technology*. 47(1): 1-18.

Mohan, C.O., Ravishankar,C.N., Srinivasa Gopal, T.K., Ashok Kumar, K. and Lalitha, K.V. 2009b. Biogenic Amines Formation in Seer Fish (*Scomberomorus commerson*) during chilled storage. *Food Research International*. 42 : 411-416.

Ozdemir, M. and Floros, J.D. 2004. Active Food Packaging

Technologies. *Critical Reviews in Food Science and Nutrition*. 44 : 185-193.

Pantazi, D., Papavergou, A., Pournis, N., Kontominas, M.G. and Savvaidis, I.N. 2008. Shelf-life of chilled fresh Mediterranean swordfish (*Xiphias gladius*) stored under various packaging conditions: microbiological, biochemical and sensory attributes. *Food Microbiology*. 25 : 136-143.

Remya, S., Mohan, C.O., Ravishankar, C.N., Sivaraman, G.K., Jha, A.K and Venkateshwarlu, G. 2018. Effect of active packaging atmosphere on the shelf life of chilled stored steaks of barracuda *Sphyraenajello*. *Indian Journal of Fisheries*. 65(4): 109-115.

Remya, S., Mohan, C.O., Venkateshwarlu, G., Sivaraman, G.K. and Ravishankar, C.N. 2016. Combined effect of O_2 scavenger and antimicrobial film on shelf life of fresh cobia (*Rachycentron canadum*) fish steaks stored at 2 °C. *Food Control*. 71 : 71-78.

Rooney, M.L. 1995. Overview of active food packaging. In: Rooney ML, editor. Active food packaging. Glasgow: Blackie Academic & Professional. 1-37.

Sankar, T.V. 2001. Changes in biochemical composition in Indian major carps in relation to size. *Fishery Technology*. 38 (1) : 22-27.

Solanki, J.B., Zofair, S.M., Remya, S. and Dodia, A.R. 2019. Effect of active and vacuum packaging on the quality of dried sardine (*Sardinella longiceps*) during storage. *Journal of Entomology and Zoology Studies*.**7**(3): 766-771.

Tarlagdis, B.G., Watts, B.M. and Younathan, M.T. 1960. A distillation methodology for the quantitation, determination of malondialdehyde in rancid foods. *The Journal of American Oil Chemist's Society*. 37 : 44-48.

Viji, P., Panda, S.K., Mohan, C.O., Bindu, J., Ravishankar, C.N. and Srinivasa Gopal, T.K. 2016. Combined effects of vacuum packaging and mint extract treatment on the biochemical, sensory and microbial changes of chill stored Indian mackerel. *Journal of Food Science and Technology*. 53(12) : 4289-4297.

Zhang X., Huang, W. and Xie, J. 2019. Effect of different packaging methods on protein oxidation and degradation of Grouper (*Epinepheluscoioides*) during refrigerated storage. *Foods*. 8(8) : 1-16.