Effect of different packaging on the shelf life of silver carp *(Hypophthalmichthys molitrix)* fillets stored at 4 ºC

R Rahmatipoor[1], L Roomiani[2*], A Askary Sary[2]

[1] Department of Food Science and Technology, Ahvaz Branch, Islamic Azad University, Ahvaz, Iran.

[2] Department of Fisheries, Ahvaz Branch, Islamic Azad University, Ahvaz, Iran

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Abstract

Freshness is one of the main quality attributes for processing, marketing and consumption of fish. Fish is increasingly becoming the favored food of people in many countries as it is rich in proteins. However, the disadvantage associated with broader consumption of fish products is their comparatively short shelf-life. Therefore, effective methods for extending shelf-life and improving quality of fresh silver carp fillets are necessary. The goal of this study was to evaluate the biochemical and microbial changes of silver carp fillets in different packaging including aerobic, vacuum and modified atmosphere packaging (MAP) when stored at 4ºC for 15 days. Data obtained from these tests was averaged and subjected to the analysis of variance (ANOVA) using SPSS software. The fillets packaged at MAP had significantly ($p<0.05$) lower pH, TVB-N, TBA and PV contents than those packaged at vacuum and aerobic packaging. The increase in viable bacterial population was significantly ($p<0.05$) higher in samples packed at vacuum and aerobic. The results showed MAP combined with refrigerated storage resulted in an extension of the shelf-life of fillets; up to 11 days at MAP, 12 days at vacuum compared to 3 days in aerobic samples.

Correspondence: L Roomiani, Department of Fisheries, Ahvaz Branch, Islamic Azad University, Ahvaz, Iran (e-mail: l.roomiani@yahoo.com).

Keywords: Modified atmosphere packaging, Vacuum packaging, Shelf-life, *Hypophthalmichthys molitrix.*

Introduction

Fish products are highly perishable food sources since the odor, flavor, color and texture are quickly altered by the growth of microorganisms, thus determining quality losses of fish meat (Hernández, Jr, Joele, Araújo & Lourenço 2017). The shelf life is defined by the period in which a food product remains safe for consumption by maintaining appropriate microbiological, physicochemical, and sensorial characteristics (Baldwin 2012). The short shelf-life of fresh fish and fish products is brought about biological reactions such as lipid oxidation, enzymatic and

microbial activities. The inherent susceptibility to deterioration of fish and fish products makes them more susceptible to food-borne hazards. Therefore, effective methods for extending shelf-life and improving quality of fresh fillets are necessary. To slow microbial growth and enzymatic processes, meat and fish are cooled during storage. In addition, the meat and fish are packaged in an atmosphere devoid of gas or with an altered gas composition, i.e. MAP (Haute, Raes, Devlieghere & Sampers 2017).

Modified atmosphere packaging (MAP), refrigerator storage and vacuum packaging can increase the shelf life of seafood and their products (Bono, Badalucco, Cusumano & Palmegiano 2012; Ginson, Kamalakanth, Bindu, Venkateswarlu, Das & Chauhan 2013; Yesudhason, Lalitha, Srinivasa Gopal & Ravishankar 2014; Kachele, Zhang, Gao & Adhikari 2017). MAP is a packaging technology that modifies or alters the gas compositions around packages to provide an atmosphere for increasing shelf life and maintaining the quality of food (Han 2005). MAP prolongs the shelf life aquatic animals by inhibiting microorganism, especially bacterial. The first reported in the early 1930s in the UK, USA and Russia on seafood in CO_2 stored (Stansby & Griffiths 1935). Packing foods in a modified atmosphere can increase the shelf life of the sea food products by approximately 40% (Coles & Kirwan 2011). A few studies have been done about the packaging fish and maintenance procedure. Based on the records of modified atmosphere packaging (MAP) on rainbow trout have done by (Kocatepe, Turan, Altan, Keskin & Ceylan 2015), sardin fish by (Ozogul, Polat & Ozogul 2004), Atlantic farmed salmon by (Fernandez, Aspe & Roeckel 2009), that indicate the positive effects of maintenance and increase their shelf-life.

Carp is one of the most widely cultured and traded species all over the world due to its fast growth rate, easy cultivation, high feed efficiency ratio and high nutritional value. Silver carp (*Hypophthalmichthys molitrix*) cultured fish is cultured with the ratio of 60 percent compared to other species in warm water ponds. The aim of this study was to investigate chemical and bacterial load changes of vacuum and modified atmosphere packaging (MAP) methods of silver carp fillet on the shelf-life of fish stored at refrigerator temperature.

Materials and Methods

Fresh silver carp was purchased from a local fish market. Each of the silver carps was 1.5–2 kg in weight. The fish was kept in ice throughout transportation to the laboratory. Fillets were obtained by removing the head and bone of fish. These fillets rinsed with 1% chlorinated water (sterile water). The fillets were divided into three packaging groups 1: aerobic (packaged in the presence of air); 2: vacuum packaged (air absence) and 3: packaged in MAP (55% N_2: 45% CO_2: 5% O_2) by using a Multivac Packaging machine (TRAY PACKING VNIT, P106, Italy). All of the packed fillets were stored at 4 °C for 15 days and in duplicate were subjected to microbial and chemical analyzes on the 0, 3, 6, 9, 12 and 15th days of storage.

pH test

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5 g of fish muscle was weighed and well mixed in 45 mL of deionized water (pH = 7) and then recorded through a digital pH meter (Metrohm, 713 pH Meter- Herisau Switzerland).

Measurement of TVB-N

Total volatile base nitrogen (TVB-N) was determined by steam distillation has measured by Kjeldahl titration method (AOAC 2000). Results of TVB-N content were expressed as mg of TVB-N 100 g $^{-1}$ fish muscle.

TBARS value test

10 g was thoroughly homogenized with 100 mL of distilled water and 2.5 mL of HCL (4m). The mixture was subjected to the distillation process for 10 minutes. The obtained liquid (5 mL) was added to 4 mL of a solution containing 0.0288 g thiobarbituric acid and 90% acetic acid. The mixture was heated in a boiling water bath (95-100 ºC) for 10 minutes and then cooled and centrifuged at 3600g at 25 ºC for 20 min. TBARS was measured at 538 nm, where D is the absorbance of the solution against the blank sample prepared by adding 5 mL of distilled water and 5 mL of TBA solution (Fan, Chi & Zhang 2008; Kilinc, Cakli, Dincer & Cadun 2007).

TBARS (μM of malonaldehyde) = 7.8×D

Measurement of PV

PV was determined according to the method presented by AOAC (2005). The results of PV are expressed as mEq kg $^{-1}$ lipids.

$$PV = \frac{(\text{thiosulfat consumption} \times \text{normality} \times 100)}{(\text{oil sample weight})}$$

Microbiological analysis

For Total Variable Count, treatments were prepared according to standard No. 2394-1(Iranian National Standard 2394-1, 2000).

Statistical analysis

Statistical analysis obtained with 3 replicates that were performed by SPSS 17 software. To assess the presence or absence of a significant difference between the values of each index in three packaging fish, ANOVA (One-way) was used and for mean comparison (Duncan test) was used and the significance level was 0.5.

Results

pH

At baseline, pH changes no significant difference between treatments (P>0.05) but, on the third day there was a significant difference among all treatments (P<0.05). On the sixth day there was significant difference between aerobic and vacuum packaging with modified atmosphere treatment (P<0.05). In ninth day there was significant difference among all treatments (P>0.05). On the twelfth day treatment, between vacuum packaging and modified atmosphere packaging with aerobic treatment packaging there was observed significant difference (P<0.05). The highest and lowest amount of pH, on the fifteenth day on the aerobic packaging (6.57 ± 0.05), and on zero

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day in aerobic packaging was (6.10 ± 0.02), respectively (Figure 1). The highest pH value was observed for the aerobic packaging followed by vacuum and MAP packaging.

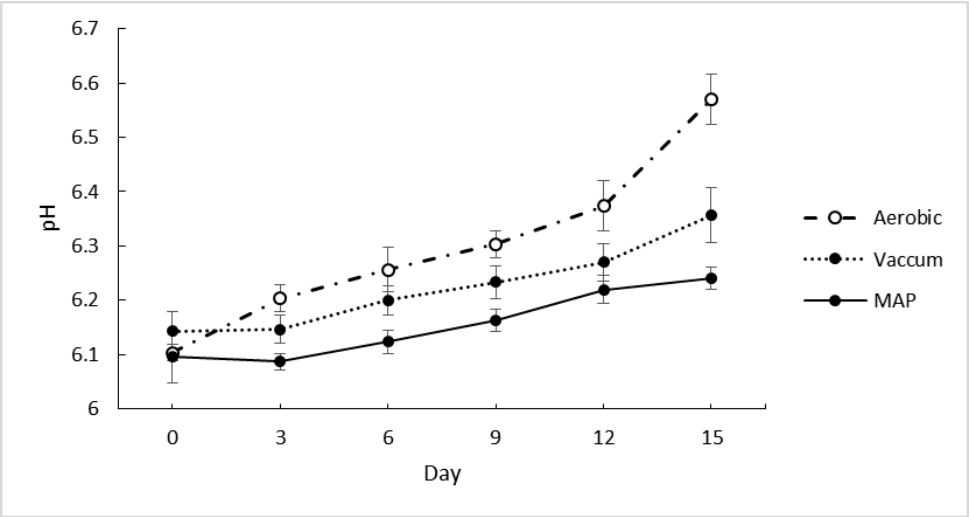

Figure 1 Comparison pH in different packaging of silver carp at refrigerator temperature.

PV

The peroxide (PV) had no significant differences at baseline between treatments (P>0.05). On the third day treatment with vacuum and modified atmosphere packaging significantly difference was observed with aerobic packaging (P<0.05). On the sixth day aerobic and vacuum packaging treatment with modified atmosphere there was significant difference (P<0.05), but on the ninth, twelfth and fifteen between all studied treatment were significant differences (P<0.05). The results of peroxide (PV) had rising process in all treatments. The highest and lowest amount of peroxide (PV) was on the fifteenth day in the aerobic packaging (9.78 ± 0.32 mEq O_2 Kg^{-1}), and on based line day in modified atmosphere packaging treatment (0.52 ± 0.08 mEq O_2 Kg^{-1}) (Figure 2).

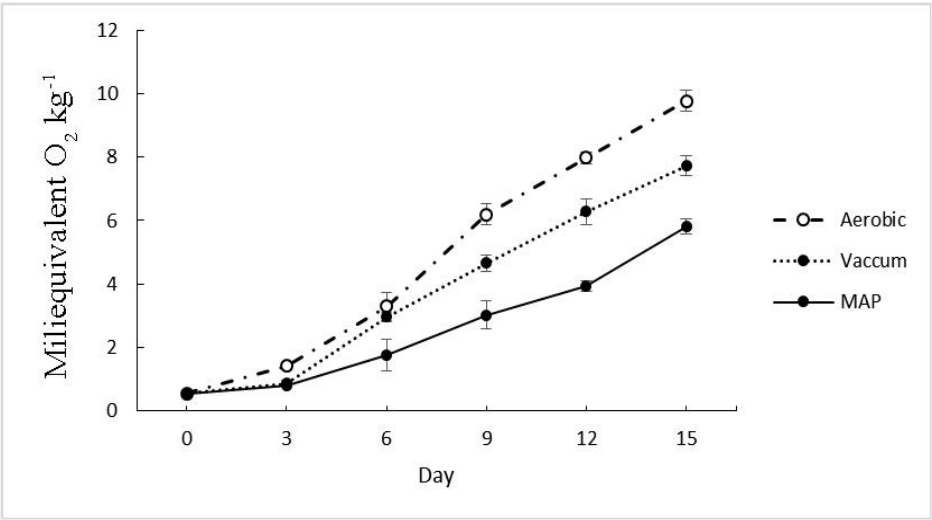

Figure 2 Comparison amount of peroxide (PV) in different packaging of silver carp at refrigerator temperature.

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TVB- N

The volatile nitrogenous based (TVB-N) at baseline there was no significant difference between treatments (P>0.05). On the third day in a vacuum and modified atmosphere packaging treatments with aerobic and packaging there was significant difference (P<0.05). On the sixth, ninth, twelfth and fifteenth day there was significant difference between all studied treatments (P<0.05). Changes TVB-N showed that from the zero to fifteenth day had rising process and in all treatments was the highest on the fifteenth day. The highest and lowest amount of TVB-N was on the fifteenth day on the aerobic packaging (32.42 ± 0.99 mg N 100 g^{-1}) and on zero day in vacuum packaging treatment (8.15 ± 0.28 mg N 100 g^{-1}) (Figure 3).

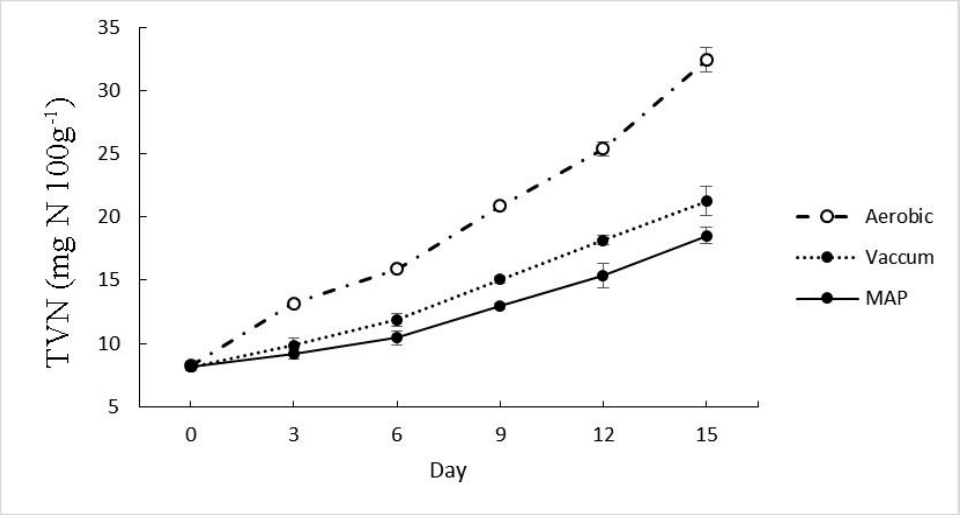

Figure 3 Comparison amount of the (TVB-N) in different packaging of silver carp at refrigerator temperature.

TBA

The thiobarbituric acid (TBA) on days zero and the third was not significantly different between treatments (P>0.05). On the sixth and ninth of vacuum and modified atmosphere packaging with aerobic packaging treatment there was observed significant difference (P<0.05). On twelfth and fifteenth day there was significant difference between all studied treatments (P<0.05). The highest and lowest amount of TBA was on the fifteenth day of aerobic packaging (2.84 ± 0.08 mg MA kg^{-1}) and on zero day in vacuum packaging treatment (0.33 ± 0.11 mg MA kg^{-1}) (Figure 4).

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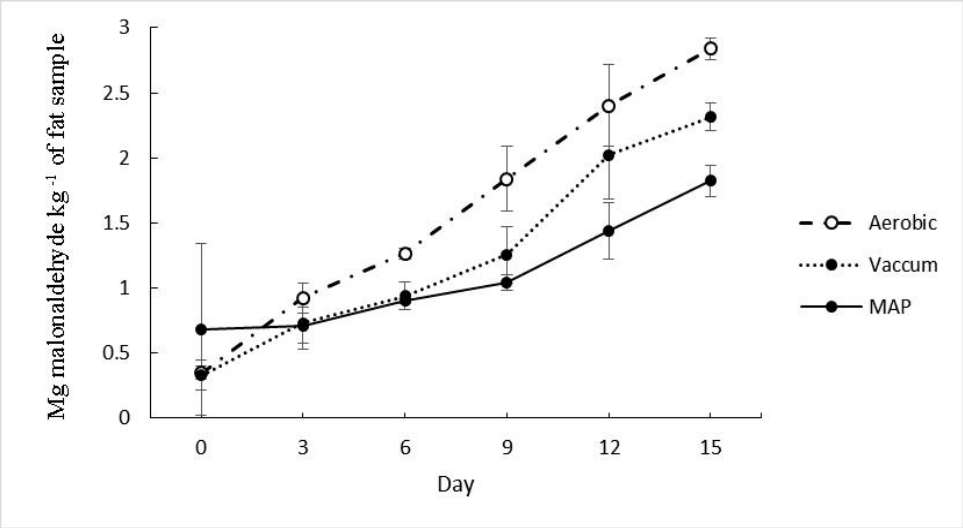

Figure 4 Comparison amount of the (TBA) in different packaging of silver carp at refrigerator temperature.

TVC

The changes in mesophilic bacteria counts are presented in Fig. 5. A according to the results, the average counts of aerobic mesophilic bacteria in all the methods, with increasing storage time at refrigerator had increased. The counts of mesophilic bacteria showed no significant differences at baseline between treatments (P>0.05). On the third, sixth, ninth, twelfth and fifteenth day significant differences were observed in vacuum and MAP with aerobic packaging (P<0.05). After 6 and 12 days in air and vacuum packaging mesophilic bacteria counts reached above than 107 CFU g $^{-1}$, but in MAP at 15th day of storage bacteria counts not reached above than 10^7 CFU g $^{-1}$. The highest and lowest counts of mesophilic bacteria orderly in the fifteenth day of aerobic packaging (9.16 ± 0.36 log CFU g^{-1}) and on zero day in vacuum packaging was (3.90 ± 0.44 log CFU g^{-1}) (Figure 5).

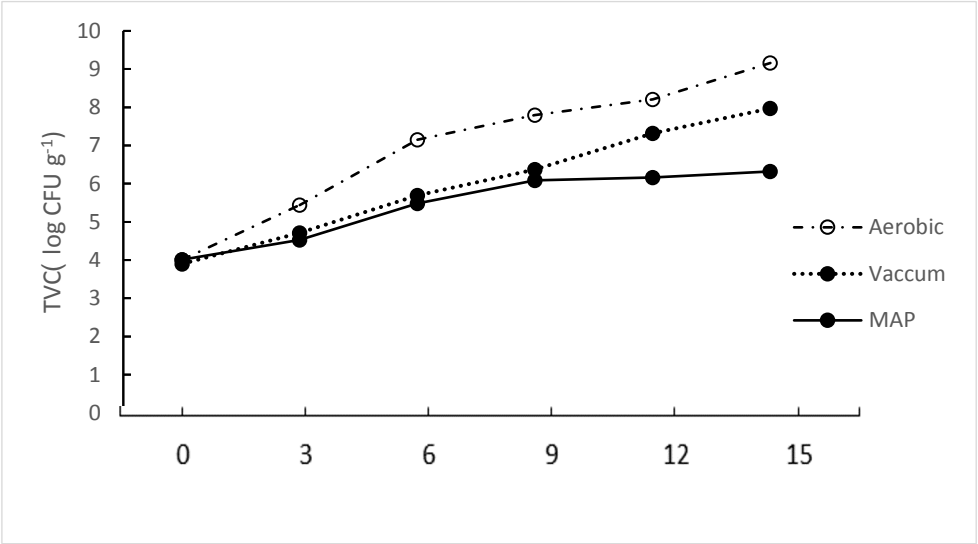

Figure 5 Changes in mesophilic bacteria counts in different packaging of silver carp at refrigerator temperature.

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Discussion

In this research, pH fillet of packed fish in modified atmosphere packaging (MAP) and vacuum was lower compared with aerobic packaging. According to the obtained data, the pH on baseline day in modified atmosphere packaging slightly decreased but then increased. A research (Rodrigues, Alvares, Sampaio, Cabral, Araujo, Franco, Mano & Junior 2016) observed that changes in pH values in all packaging conditions except the control group that did not alter ($P>0.05$) throughout the storage period (22 days).

In study of (Arashisar, Hisar, Kaya & Yanik 2004), the highest average pH was observed on day 12. Results of (Arkoudelos, Stamatis & Samaras 2007) revealed that in all packaging treatments (air, vacuum, MAP) the value of pH no statistically significant ($P<0.05$). Fish pH is one of the factors that change after the death of fish during the storage. Most fish have a small amount of carbohydrates in their muscle tissue (lower than 5%), that after their death only a small amount of lactic acid is produced as glycolysis and pH of fish meat slightly reduced. But during storage, the amount of pH is increased because it is possible to produce compounds such as ammonia, trimethylamine, dimethylamine and as well as other biological amine that are produced by spoilage bacteria in fish (Mendes & Goncalves 2008). In MAP packaging CO_2 solubility and absorption in muscle where it is converted in carbonic acid leading to acidification and decrease of pH, that observed by (DeWitt & Oliveira 2016). The pH is one of the factors affecting microbial growth and food spoilage and yet can be influenced by microbiologic activity. The pH of fish muscle generally is 6.7-7 that change with the season, nutrition and fish temperature. The pH more than 7 indicates corruption (Masniyom, Soottawat & Visessanguan 2005).

To determine the primary product of lipid oxidation, peroxide index is measured. Since peroxides don't make change in the fish sensory properties and are without tastes and odor compounds can't be detected by consumers. However, these compounds cause the secondary compounds such as aldehydes and ketones which are rapid the oxidation (Stamatis & Arkoudelos 2007). Peroxide is an unstable compound that will eventually become malondialdehyde that this material can establish with the amino acid crosslinking and the result is production of amine bonds (Cakli, Kilinc, Dincer & Tolasa 2006).

Hydro-peroxides are primary products of oxidation and polyunsaturated fatty acids (Goulas & Kontominas 2006). The peroxide (PV) on fifteenth day of the vacuum and modified atmosphere packaging (MAP) increased but was significantly lower than control treatments ($P<0.05$). This can be due to lack of oxygen in vacuum packaging and the presence of carbon dioxide (45% in this study) in modified atmosphere packaging (MAP) and inhibit properties on the growth of bacteria. Because of carbon dioxide gas solubility in water and lipid can penetrate into the core of the microorganism nucleus and reduce the nucleus

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pH, thereby slowing down the growth and development of microorganisms and, thus to some extent can affect the amount of peroxide production of bacterial enzymes and reduced it (Cakli *et al*., 2006), and as in modified atmosphere packaging (MAP) nitrogen gas is used, this gas delays oxidation rapid and accordance with the finding of other researches (Anelich, Hoffman & Swanepoel 2001; Fagan, Gormley & Ui Mhuircheartaigh 2004; Duun & Rustad 2008). The limit peroxide for human consumption fillet is 10 mM O2 kg^{-1} of lipid (Lodasa, Barros-Velazquez, Gallardo & Aubourg 2004). However, (Binsi, Viji, Visnuvinayagam, Ninan, Sangeeta, Triveni & Ravishankar 2015) considered that PV value of 20 mEq O_2 kg^{-1} of fat necessary for oils to become rancid. The result obtained in this study is lower than accepted level.

At the beginning of storage, the TVB-N value was 8 mg 100 g^{-1} for fish fillets in all packaging treatments. TVB-N values were above 30 mg 100 g^{-1} in aerobic packaging, 20 mg 100 g^{-1} in vacuum packaging and 10 mg 100 g^{-1} on day 15, that the statistical analysis of the TVB-N data showed that significant difference ($P<0.05$). The study data show that the samples which are packed in MAP the TVB-N had slower increase. Due to the presence of carbon dioxide gas which is partly prevent the growth of spoilage bacteria and delay their growth. The TVB-N in samples packaged in vacuum increased slower than aerobic packaging that was due to the anaerobic environment inside the package which delays the growth of spoilage bacteria. The TVB-N usually content of trimethylamine and ammonia that is produced by bacterial spoilage, which are often used as an indicator to assess quality and shelf-life ability of fishery products. TVB-N was compounds that found in small amount in fresh fish and increased by adding storage time. However, TVBN can´ not be used to estimate the amount of freshness degree in the early stages, and only used to estimate the amount of not fresh fish (Masniyom *et al*., 2005; Kilinc, Cakli, Dincer & Cadun 2007). The cause TVB-N in meat is enzymes and microorganisms activity. TVB-N value depends on the species fish feeding, season, size and others environment parameters (Binsi *et al*., 2015). The acceptable range of expressed TVB-N index is different in studies. For Nile perch (Amegovu, Sserunjogi, Ogwok & Makokha 2012) TVB-N values of 25 $mg100g^{-1}$ has been suggested, but common carp packed in chilled was 15 mg $100g^{-1}$ under MAP packaging (Jezek & Buchtova 2010), for gutted freshwater pabda fish 18-20 mg 100 g^{-1} in vacuum (Binsi *et al*., 2015) and in our study was 15-20 mg 100 g^{-1} in vacuum and MAP packaging. TVB- N for human consumption is less than 20 mg $100g^{-1}$ (Jezek & Buchtova 2010). The minor increase of the amount of volatile nitrogen bases in the early stages in due to decomposition of amino acids and nucleotides, while increasing the amount of volatile nitrogen bases in the final stages are due to increased microbial activity.

In this study, all treatments show the thiobarbituric acid (TBA) changes that from the baseline to the fifteenth the amount of this index had increased process and in vacuum and modified atmosphere packaging (MAP) was significantly lower than the control group

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($P<0.05$). TBA test often used to determine the degree of lipid oxidation in fish. Fat oxidation is related to oxidation of polyunsaturated fatty acids in fish muscle, that cause odor and bitter taste in fish thus shortening the shelf life (Silva, Prinyawiwatkul, King, Kyoon, Bankston & Ge 2008). TBA index is to measure an amount of malonaldehyde which that is the secondary product of the oxidation of polyunsaturated fatty acids (Bremner 2002). TBA is the product obtained from the second stage of the auto-oxidation in which peroxide change to materials such as aldehydes and ketones (Feliciano, Lee, Lopes & Pascall 2010). Increased amount of TBA during refrigerated storage maybe caused by partial hydrogenation of fish tissue and increasing the oxidation of unsaturated fatty acids (Silva *et al*., 2009). In a study of gutted European sea bass fillets in modified atmosphere, the TBA value increased during storage (Parlapani, Haroutounian, Nychat & Boziaris 2015). Similar result has been reported (Castro, Padron, Cansino, Velazquez & De Larriva 2006).

Permitted level of TBA fish meet is 1-2 mg MA kg^{-1} (Lakshmanan, Shakila & Jeyasekaran 2002). Our results showed that TBA value has crossed 2 mg MA kg^{-1} on 9th, 12th and 15th day in air, vacuum and MAP packaging, respectively.

Microorganisms are one of the main reasons for seafood spoilage. In this study, the counts of mesophilic bacteria increased over time of storage. In MAP packaging, in end of storage (15th day), mesophilic bacteria counts not reached above than 10^7 log CFU g^{-1} (the highest acceptable limit), but in vacuum 6 days more than air packaging reached to 10^7 log CFU g^{-1}. Effects of CO_2 in MAP packaging, which has bacteriostatic properties and easily dissolved in water in the tissue and accumulation carbonic acid decrease the pH and increase the acidity of the solution, could be an explanation for this situation. The mesophilic and psychrotrophic bacteria count in fillets of rainbow trout under different MAP condition (100% CO_2, 2.5% O_2 + 7.5% N_2 + 90% CO_2 and 30% O_2 + 30% N_2 + 40% CO_2) increase with length of storage at 4 °C. In their study, effects of 100 CO_2 on bacterial growth were higher than that of the 90% and 40% CO_2 (Arashisar *et al*., 2004). In study (Bouletis, Arvanitoyannis, Hadjichristodoulou, Neofitou, Parlapani & Gkagtzis 2016) found that shelf life cuttlefish reached 2 (air), 2 (vacuum), 4 (MAP: 20% CO_2: 80% N_2), 8 (50% CO_2: 50% N_2) and 8 (70% CO_2: 30% N_2) days. They revealed that packaging with high CO_2 different significantly from the air on the 6th day of storage ($P<0.05$). In our study, trend in TVC indicated a positive correlation with TVB-N (r = 94%) that is produced by autolytic and bacterial degradation. Mesophile bacteria count results showed that high CO2 in MAP treatments different significantly from the control from 6th day of storage onwards, that is inconsistent with this research. CO_2 delayed spoilage of sea food by inhibiting of other foodborne bacteria (Gram negative, aerobic and psychrotrophic bacteria).

Conclusions

Based on microbial and biochemical indices degradation, MAP packaging was the most effective treatment for the preservation of silver

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carp (*Hypophthalmichthys molitrix*) fillets in refrigerated temperature. Survey results have shown that samples of silver carp fillet with vacuum packaging has the capacity of 9 days storage in refrigerator. While the samples packed in modified atmosphere (MAP) has the ability to hold up to 15 days.

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اثر بسته‌بندی‌های مختلف بر ماندگاری فیله ماهی کپور نقره‌ای نگهداری شده در ۴ درجه سانتی‌گراد

ریحانه رحمتی پور[۱]، لاله رومیانی*[۲]، ابوالفضل عسکری ساری[۲]

[۱] دانشجوی کارشناسی ارشد گروه علوم و صنایع غذایی، واحد اهواز، دانشگاه آزاد اسلامی، اهواز، ایران
[۲] گروه شیلات، واحد اهواز، دانشگاه آزاد اسلامی، اهواز، ایران

چکیده

تازگی یکی از مهم‌ترین نشانه‌های کیفیت برای فرآوری، بازاریابی و مصرف ماهی است. پروتئین موجود در ماهی باعث شده که به یک غذای مطلوب برای مردم جهان تبدیل شود. هرچند مصرف گسترده محصولات شیلاتی با معایبی همراه است که ماندگاری کوتاه چنین محصولاتی می‌باشد. بنابراین روش های موثر برای افزایش ماندگاری و بهبود کیفیت فیله‌های ماهی کپور نقره‌ای *(Hypophthalmichthys molitrix)* ضروری است. هدف این مطالعه ارزیابی تغییرات بیوشیمیایی و میکروبی فیله ماهی کپور نقره‌ای در بسته‌بندی‌های متفاوت شامل هوازی، خلاء و اتمسفر تغییر یافته در ۴ درجه سانتی‌گراد می‌باشد. داده‌ها در ANOVA با استفاده از نرم افزار SPSS مورد تجزیه و تحلیل قرار گرفتند. فیله‌های بسته‌بندی شده در اتمسفر تغییر یافته در مقایسه با بسته‌بندی هوازی و خلاء دارای pH، TVB-N، TBA و PV کمتری بودند ($P<0.05$). افزایش بار میکروبی بطور معنی‌داری بیشتر از نمونه‌های خلاء و هوازی بود ($P<0.05$). نتایج نشان داد که بسته‌بندی اتمسفر تغییر یافته در ترکیب با دمای یخچالی باعث افزایش ماندگاری فیله‌ها گردید بطوریکه در بسته بندی اتمسفر تغییر یافته تا ۱۱ روز، در خلاء تا ۱۲ روز در مقایسه با نمونه‌های هوازی ۳ روز مشاهده گردید.

کلمات کلیدی: بسته‌بندی اتمسفر تغییر یافته، بسته‌بندی خلاء، ماندگاری، کپور نقره‌ای

***نویسنده مسئول:** l.roomiani@yahoo.com

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