

## Effects of vacuum and modified atmosphere packaging on the shelf life of Rohu fish (*Labeo rohita*) stored at refrigerated temperature (4°C)

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### Abstract

Vacuum packaging and modified atmosphere packaging (MAP) are important techniques used for the extension of fish and fish product's shelf life. The present work evaluated the quality and shelf life of Rohu fish (*Labeo rohita*) by biochemical and microbiological analyses under different packaging types namely (1) unsealed pack (control), (2) vacuum pack, (3) MAP-1 (50% CO<sub>2</sub> and 50% N<sub>2</sub>), and (4) MAP-2 (50% CO<sub>2</sub> and 50% O<sub>2</sub>) at 3-day intervals during 18 days of refrigerated storage (4°C). Result showed that pH, total volatile base nitrogen (TVB-N), and thiobarbituric acid reactive substances (TBARS) values for all packaging conditions were within the acceptable limit during the storage period, except for TBARS value in MAP-2 sample. The total viable count (TVC) gradually increased with the progress of time in all packaging conditions. However, the TVC values were significantly ( $p < 0.05$ ) lower on 9<sup>th</sup> and 12<sup>th</sup> day of storage in MAP samples as compared to that of the control sample. Considering the total bacterial counts, 7 log CFU/g, the shelf life was determined at approximately 8, 11, 13, and 16 days for control, vacuum pack, MAP-1, and MAP-2 sample, respectively. Therefore, MAP is recommended to be used to display and preserve fishes in the superstores.

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### Introduction

Rohu fish (*Labeo rohita*), also known as Indian major carp (order: Cypriniformes), is available in the riverine system of Bangladesh. It is abundant throughout the country, and preferred as an aquaculture species for its high environmental tolerance, disease resistance, faster growth, and high flesh content. The fat of cultured Rohu fish is rich with various vitamins (e.g., A, D, E, and K) as well as high content of saturated and monounsaturated fatty acids. In contrast, Rohu fish from wild sources contain higher levels of polyunsaturated fatty acids (Sharma *et al.*, 2010). Rohu fish have also been reported to have high content of minerals namely calcium, zinc, iron, and thallium (de Silva and Anderson, 1994).

Maintaining the cold chain is always a big concern to maintain the quality and maximise the shelf life of fish. In Bangladesh, icing, chilling, and freezing are the most common techniques used in low-temperature preservation. Nowadays, residents living in cities and mega-cities, particularly corporate officials and busy homemakers, prefer ready-to-cook (RTC) foods over cooking raw foods to save time. Therefore, there is a high demand for an extended shelf life of fish for

retailing purposes. Icing and refrigerated storage can extend the shelf life of fish and fishery products for 2 - 14 days depending on the raw material quality (Stammen *et al.*, 1990). Sound food packaging is a prerequisite to protect food from foodborne pathogens, spoilage microorganisms, and oxidation (Choi *et al.*, 2016). The quality and type of packaging materials are critical to ensure food quality and safety with extended shelf life. Modified atmosphere packaging (MAP) and vacuum packaging are two important methods to be adapted for preserving fish and fish products.

Vacuum packaging is the placement of a product inside a packaging material with low permeability of oxygen, followed by air exhaustion, and sealing (Smith *et al.*, 1990). In this type of packaging, the gaseous atmosphere is almost eliminated; therefore, changes in the gas composition are apparent, particularly during storage. These changes happen due to the microbial activity by increasing the amount of carbon dioxide (CO<sub>2</sub>; 10 - 20%), that perhaps suppress the growth of undesirable microorganisms (Silliker and Wolfe, 1980). Total removal of oxygen is crucial in oxygen-sensitive foods because the existence of O<sub>2</sub> facilitates oxidation, which leads to the accumulation of aerobic bacteria and

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moulds (Goodburn and Halligan, 1988). Vacuum packaging can hinder the aerobic bacterial growth and extend the shelf life of fish fillets (Gram and Huss, 1996). However, anaerobic bacteria can grow in the oxygen-free environment that may threaten food safety, particularly the accumulation of toxin by *Clostridium botulinum* (Pantazi et al., 2008).

On the other hand, MAP is a preservation technique that involves the alteration of the atmospheric environment around food by replacing one or a mixture of protective gases (Reddy et al., 1991; DeWitt and Oliveira, 2016). This method has been reported successful in inhibiting the spoilage and increasing the shelf life of fresh fish (DeWitt and Oliveira, 2016). In this type of packaging, after the incorporation of gas mixture in the package, no further control of gas mixture is applied. However, changes in gas mixture occurred during the progression of the storage period (Sivertsvik et al., 2002). The aim of MAP is delaying bacterial activity and chemical reactions by modifying the headspace of food products during packaging by adding various gases (DeWitt and Oliveira, 2016).

Oxygen, carbon dioxide, and nitrogen are the most commonly used gases in the MAP system. The concentration of gas mixture depends on the food items and their mechanism of spoilage that determine the shelf life (Reddy et al., 1991). The exclusion of oxygen in the package is necessary to inhibit deterioration and extending product quality during storage (Church and Parsons, 1995). The most widely used gas for MAP for fishery products is CO<sub>2</sub> that inhibits microbial growth. The inhibition of microbial growth depends on the concentration of CO<sub>2</sub> as the growth of aerobic microorganisms such as *Shewanella putrefaciens* and *Pseudomonas* spp. can be delayed by CO<sub>2</sub> (Sivertsvik et al., 2002). The growth phase of microorganisms is affected by the effectiveness of CO<sub>2</sub>. Due to the increased CO<sub>2</sub> concentrations, the lag phase period increases, and the exponential growth rate decreases during the logarithmic phase of microorganisms (Farber, 1991). N<sub>2</sub> is commonly used as a filler or balance gas substituting O<sub>2</sub> in the modified atmosphere packages. However, N<sub>2</sub> acts as an alternative for vacuum packaging when the product is delicate, or else to end pack collapse resulted from the absorption of CO<sub>2</sub> (Church and Parsons, 1995). O<sub>2</sub> is also sometimes used in MAP systems mainly to counter the effects of anaerobic or micro-aerophilic organisms and non-oxidative reactions. It has been found that O<sub>2</sub> can hamper the growth of anaerobic bacteria as well as the accumulation of toxin by *Clostridium botulinum* type E (Pantazi et al., 2008). Another reason for using O<sub>2</sub> is to maintain the colouring pigment intact in meat or flesh, which is preferred by consumers

(Reddy et al., 1991).

MAP has been studied mostly in marine fish (Reddy et al., 1991; DeWitt and Oliveira, 2016). Nevertheless, very few studies have been found of MAP on freshwater fishes such as tilapia (*Oreochromis niloticus*), sutchi catfish (*Pangasius hypophthalmus*), and common carps (*Cyprinus carpio*) (Noseda et al., 2012; Babic et al., 2015; DeWitt and Oliveira, 2016). This packaging system has not yet been introduced for the preservation of fishes in developing countries, including Bangladesh. MAP provides several advantages like high-quality fish fillets with an extended shelf life, good hygienic standards, and most importantly, food safety. As the demand for quality food with a prolonged shelf life is increasing, modern technologies like vacuum and modified atmosphere packaging have a high prospect. Therefore, the present work aimed to evaluate the quality and to determine the shelf life of packaged sliced Rohu fish under different packaging conditions stored at refrigerated temperature (4°C).

## Materials and methods

### Collection and preparation of samples

The Rohu fish samples with an average size of  $1.8 \pm 0.3$  kg were purchased alive from a local market, and immediately transported to the Laboratory of Quality Control, Department of Fisheries, University of Rajshahi. Upon arrival and once dead, fish samples were then washed with running tap water, and cut into small slices with an average weight of  $100 \pm 10$  g. Then, the sliced Rohu fish samples were washed twice with running tap water, and finally with distilled water.

### Packaging and storage of samples

Around 200 g of sliced Rohu fish samples were packed under vacuum and modified atmosphere packaging in low moisture and gas permeable plastic pouch. Multi-layered (Polythene/Polyamide/Polythene) transparent pouch with 100 µm density was used as the packaging material. Four types of packaging were applied using different gas mixture by following the method of Noseda et al. (2012). These four types of packaging were: (1) aerobic, unsealed pack as control; (2) vacuum pack; (3) MAP-1 with 50% CO<sub>2</sub> and 50% N<sub>2</sub>, and (4) MAP-2 with 50% CO<sub>2</sub> and 50% O<sub>2</sub>. Vacuum packaging and MAP were prepared by the packaging machine (C-100, Multivac, Germany) which was attached with a gas mixer (KM100-3MEM, WITT, Germany) following the guidelines of the manufacturer. Monitoring of the O<sub>2</sub>, N<sub>2</sub>, and CO<sub>2</sub> levels in the headspace of packaged

samples was done with a gas analyser (Oxybaby M+, WITT, Germany). All the samples were stored in the laboratory refrigerator (GL-C322RLBB-PZ, LG, South Korea) at 4°C. Three samples (as replication) from each of the control and treatments were analysed at 3-day intervals during the 18 days of storage period.

#### Biochemical and microbiological analyses

Various microbiological and biochemical parameters were analysed to determine the shelf life of the sliced Rohu fish samples stored at refrigerated temperature. Ten grams of the cut fish flesh was homogenised with a mixer grinder (REX 500, Bajaj, India) after adding 50 mL of distilled water, and then the pH of the homogenate was determined by a pH meter (HI2002 Edge, Hanna Inst, USA). Total volatile base nitrogen (TVB-N) was measured according to the European Commission Regulations (EC, 2005) method. Thiobarbituric acid reactive substance (TBARS) was estimated by a colorimetric method according to Witte *et al.* (1970). Total viable count (TVC) was determined on plate count agar (Sigma-Aldrich, USA) by standard pour plate count method following the decimal dilution technique by American Public Health Association (APHA, 1992), and was expressed as colony-forming units (CFU/g). All plates were incubated at 35°C for 48 h in an incubator (Poleko, Poland), and later the colonies were counted. Bacterial counts were then converted into logarithms.

#### Statistical analysis

One-way analysis of variance (ANOVA) was performed, and the differences among treatments were determined by Tukey's test using SPSS-20 software.  $p < 0.05$  was considered statistically significant.

## Results

#### pH value

The initial pH value of sliced Rohu fish samples was 6.50. Then, the pH value gradually decreased until 3<sup>rd</sup> day for control, 6<sup>th</sup> day for vacuum packaging and MAP-2, and 9<sup>th</sup> day of storage for MAP-1 samples, respectively, and then showed an increasing trend with some fluctuations (Table 1). No significant ( $p > 0.05$ ) differences were observed in pH values in all four packaging conditions from start to end of the storage period. However, MAP-1 and MAP-2 samples showed a gradual decrease in pH value until the 9<sup>th</sup> day of storage, followed by a gradual increase until the end of the storage period. Relatively lower pH values were found in MAP-1 as compared to MAP-2 (Table 1).

#### Total volatile base nitrogen (TVB-N) value

The initial TVB-N value was 1.18 mg/100 g in sliced Rohu fish sample, and then a gradual increase was observed during the rest of the storage period. The highest TVB-N value was found at 6.04 mg/100 g on the 18<sup>th</sup> day for MAP-1 sample. The TVB-N values ranged from 1.18 - 5.44, 1.18 - 6.04, and 1.18 - 5.46 mg/100 g for vacuum packaging, MAP-1, and MAP-2 samples, respectively (Table 2). In all four packaging conditions, no significant differences ( $p > 0.05$ ) were observed until the 6<sup>th</sup> day of storage (Table 2). However, significantly ( $p < 0.05$ ) lower TVB-N values were revealed at 9<sup>th</sup> day for MAP-1 and MAP-2 samples as compared to control. It was evident that MAP samples showed a slower increase in TVB-N value.

#### Thiobarbituric acid reactive substances (TBARS) value

The initial TBARS value was found at 0.23 mg malonaldehyde/kg from sliced Rohu fish

Table 1. pH values of sliced Rohu fish under different packaging conditions at refrigerated storage (4°C).

Treatment	Storage period (day)						
	0	3	6	9	12	15	18
Unsealed pack (control)	6.50 ± 0.18 <sup>a</sup>	6.14 ± 0.04 <sup>a</sup>	6.48 ± 0.04 <sup>a</sup>	6.22 ± 0.04 <sup>a</sup>	-	-	-
Vacuum pack	6.50 ± 0.18 <sup>a</sup>	6.25 ± 0.05 <sup>ab</sup>	6.24 ± 0.01 <sup>a</sup>	6.36 ± 0.09 <sup>a</sup>	6.60 ± 0.06	-	-
MAP-1 (50% CO <sub>2</sub> and 50% N <sub>2</sub> )	6.50 ± 0.18 <sup>a</sup>	6.35 ± 0.07 <sup>ab</sup>	6.16 ± 0.10 <sup>a</sup>	6.12 ± 0.06 <sup>a</sup>	6.39 ± 0.19	6.37 ± 0.18	6.44 ± 0.06
MAP-2 (50% CO <sub>2</sub> and 50% O <sub>2</sub> )	6.50 ± 0.18 <sup>a</sup>	6.41 ± 0.04 <sup>b</sup>	6.25 ± 0.16 <sup>a</sup>	6.27 ± 0.07 <sup>a</sup>	6.58 ± 0.02	6.38 ± 0.04	6.77 ± 0.11

Values are mean ± SD of three replicates ( $n = 3$ ). Values in the same column with different superscript letters indicate significant difference ( $p < 0.05$ ).

Table 2. Total volatile base nitrogen (TVB-N) values (mg/100 g) of sliced Rohu fish under different packaging conditions at refrigerated storage (4°C).

Treatment	Storage period (day)						
	0	3	6	9	12	15	18
Unsealed pack (control)	1.18 ± 0.48 <sup>a</sup>	2.38 ± 0.59 <sup>a</sup>	3.36 ± 0.79 <sup>a</sup>	3.90 ± 0.76 <sup>ab</sup>	-	-	-
Vacuum pack	1.18 ± 0.48 <sup>a</sup>	2.66 ± 0.99 <sup>a</sup>	3.92 ± 0.79 <sup>a</sup>	5.05 ± 0.40 <sup>b</sup>	5.44 ± 0.15	-	-
MAP-1 (50% CO <sub>2</sub> and 50% N <sub>2</sub> )	1.18 ± 0.48 <sup>a</sup>	1.82 ± 0.59 <sup>a</sup>	2.50 ± 1.16 <sup>a</sup>	2.94 ± 0.59 <sup>ab</sup>	3.30 ± 0.31	4.20 ± 0.57	6.04 ± 0.57
MAP-2 (50% CO <sub>2</sub> and 50% O <sub>2</sub> )	1.18 ± 0.48 <sup>a</sup>	2.10 ± 0.59 <sup>a</sup>	2.64 ± 0.17 <sup>a</sup>	2.66 ± 0.20 <sup>a</sup>	3.30 ± 0.71	4.62 ± 0.59	5.46 ± 0.59

Values are mean ± SD of three replicates ( $n = 3$ ). Values in the same column with different superscript letters indicate significant difference ( $p < 0.05$ ).

samples. With the progression of storage, the TBARS value slowly increased in all packaging conditions at the storage period (Table 3). Significantly ( $p < 0.05$ ) lower TBARS values were found on 3<sup>rd</sup>, 6<sup>th</sup>, and 9<sup>th</sup> day of storage in the vacuum pack and MAP-1 samples as compared to control. In addition, significantly ( $p < 0.05$ ) higher TBARS values were also determined on 3<sup>rd</sup>, 6<sup>th</sup>, and 9<sup>th</sup> day of storage for MAP-2 sample as compared to the other samples (Table 3).

#### Total viable count (TVC)

In sliced Rohu fish samples, the initial total viable count (TVC) was log 4.29 CFU/g. With the progression of storage, the TVC values of sliced Rohu fish samples gradually increased in all packaging conditions. Nevertheless, significantly ( $p < 0.05$ ) lower TVC values were recorded on the 9<sup>th</sup> and 12<sup>th</sup> day of storage in all treated samples as compared to control (Table 4).

Table 3. Thiobarbituric acid reactive substances (TBARS) values (mg malonaldehyde/kg) of sliced Rohu fish under different packaging conditions at refrigerated storage (4°C).

Treatment	Storage period (day)						
	0	3	6	9	12	15	18
Unsealed pack (control)	0.23 ± 0.11 <sup>a</sup>	0.56 ± 0.01 <sup>b</sup>	1.08 ± 0.10 <sup>c</sup>	1.17 ± 0.07 <sup>b</sup>	-	-	-
Vacuum pack	0.23 ± 0.11 <sup>a</sup>	0.41 ± 0.03 <sup>a</sup>	0.17 ± 0.06 <sup>a</sup>	0.46 ± 0.05 <sup>a</sup>	0.74 ± 0.07	-	-
MAP-1 (50% CO <sub>2</sub> and 50% N <sub>2</sub> )	0.23 ± 0.11 <sup>a</sup>	0.45 ± 0.04 <sup>a</sup>	0.53 ± 0.17 <sup>b</sup>	1.09 ± 0.02 <sup>b</sup>	0.78 ± 0.11	1.40 ± 0.05	2.70 ± 0.12
MAP-2 (50% CO <sub>2</sub> and 50% O <sub>2</sub> )	0.23 ± 0.11 <sup>a</sup>	0.56 ± 0.04 <sup>b</sup>	0.90 ± 0.12 <sup>c</sup>	2.38 ± 0.17 <sup>c</sup>	3.69 ± 0.47	3.27 ± 0.54	5.40 ± 0.17

Values are mean ± SD of three replicates ( $n = 3$ ). Values in the same column with different superscript letters indicate significant difference ( $p < 0.05$ ).

Table 4. Total viable count (TVC) (log CFU/g) of sliced Rohu fish under different packaging conditions at refrigerated storage (4°C).

Treatment	Storage period (day)						
	0	3	6	9	12	15	18
Unsealed pack (control)	4.29 ± 0.09 <sup>a</sup>	4.31 ± 0.08 <sup>a</sup>	4.67 ± 0.10 <sup>a</sup>	7.33 ± 0.45 <sup>b</sup>	8.23 ± 0.13 <sup>c</sup>	-	-
Vacuum pack	4.29 ± 0.09 <sup>a</sup>	4.18 ± 0.10 <sup>a</sup>	4.70 ± 0.25 <sup>a</sup>	6.38 ± 0.09 <sup>ab</sup>	7.33 ± 0.14 <sup>b</sup>	-	-
MAP-1 (50% CO <sub>2</sub> and 50% N <sub>2</sub> )	4.29 ± 0.09 <sup>a</sup>	5.00 ± 0.73 <sup>a</sup>	5.44 ± 0.02 <sup>b</sup>	5.70 ± 0.12 <sup>a</sup>	6.14 ± 0.07 <sup>a</sup>	6.77 ± 0.41	7.67 ± 0.16
MAP-2 (50% CO <sub>2</sub> and 50% O <sub>2</sub> )	4.29 ± 0.09 <sup>a</sup>	5.24 ± 0.01 <sup>a</sup>	5.32 ± 0.06 <sup>b</sup>	5.31 ± 0.32 <sup>a</sup>	6.64 ± 0.27 <sup>a</sup>	7.65 ± 0.04	7.49 ± 0.11

Values are mean ± SD of three replicates ( $n = 3$ ). Values in the same column with different superscript letters indicate significant difference ( $p < 0.05$ ).

## Discussion

Various parameters can be observed to evaluate the quality of fish and fishery products. Among them, pH is considered as an important parameter. In the present work, pH values of all treatments were found within the limit (6.8~7.0) of acceptability (Metin *et al.*, 2001). The lower pH value of fish packaged with MAP condition at a higher concentration of CO<sub>2</sub> was reported in several studies (Stamatis and Arkoudelos, 2007; Provincial *et al.*, 2010). In the case of vacuum packaging and MAP, lower pH value was perhaps caused by lactacidogenic bacteria, linked to the inhibition of Gram-negative aerobic bacteria (mainly pseudomonads). The Gram-negative aerobic bacteria become predominant during the storage period as their number increased (Leroi, 2010). In MAP, the initial dropped of pH value perhaps (by 6<sup>th</sup> day of storage) occurred as a result of the dissolution of CO<sub>2</sub> in the muscle tissues (Jezek and Buchtova, 2012). Moreover, the fish muscle surface absorbs CO<sub>2</sub>, thus acidifying it with the formation of carbonic acid (Banks *et al.*, 1980). It was evident in the past study that the result of increased pH at a later stage was linked with the generation of basic components such as ammonia, dimethylamine, trimethylamine, and other biogenic amines, as well as microbial spoilage (Goulas and Kontominas, 2007). The lower pH value in MAP-1 (Table 1) perhaps was reflecting the increased CO<sub>2</sub> concentration throughout the storage period.

Total volatile base nitrogen (TVB-N) is termed as the sum of ammonia (NH<sub>3</sub>), dimethylamine (DMA), and trimethylamine (TMA) in fish as a whole (Wu and Bechtel, 2008). It is commonly used as an indicator to predict the bacterial spoilage of fish. In the present work, the TVB-N values were found within the limit (30 - 35 mg/100 g) of acceptability in all packaging conditions (Huss, 1988) (Table 2). Therefore, the results of the present work indicated that all packaging systems can ensure the safe limit of TVB-N value for human consumption (Table 2). It was evident that the samples which were packed in MAP showed a slower increase of TVB-N value, which is supported by a previous study where silver carp fillets were preserved at 4°C (Rahmatipoor *et al.*, 2017). In the present work, MAP-1 (50% CO<sub>2</sub> and 50% N<sub>2</sub>) showed better performance than MAP-2 (50% CO<sub>2</sub> and 50% O<sub>2</sub>) throughout the storage period. Jezek and Buchtova (2012) also observed a similar type of results for silver carp fillets where the MAP-2 (70% N<sub>2</sub> and 30% CO<sub>2</sub>) showed better performance

as compared to MAP-1 (69% N<sub>2</sub>, 25% CO<sub>2</sub>, 5%O<sub>2</sub>, 1% CO). Besides, the better performance of MAP-1 in the present work may be due to the effect of bacteriostatic properties of CO<sub>2</sub>. A past study claimed that the presence of CO<sub>2</sub> is responsible for partial prevention and delay of spoilage bacterial growth (Farber, 1991). In the present work, the presence of O<sub>2</sub> in MAP-2 (50% CO<sub>2</sub> and 50% O<sub>2</sub>) package may predominantly accelerate the growth of aerobic bacteria and thus eventually fasten the spoilage process.

Thiobarbituric acid reactive substances (TBARS) is a popular method to evaluate lipid oxidation, and thus the quality of food. TBARS index is used to measure the amount of malonaldehyde, a secondary product of the oxidation of polyunsaturated fatty acids (Bremner, 2002), in which, modification of peroxide occurs, thus resulted in the production of materials such as aldehydes and ketones (Feliciano *et al.*, 2010). For TBARS value, the acceptable limit is 2 mg malonaldehyde/kg fish sample, and when this limit is exceeded, an obnoxious odour and taste build up will be observed in fish (Connell, 1990). In the present work, the TBARS values in all packaging conditions were within the acceptable limit throughout the storage period, except the MAP-2 sample. The TBARS value of MAP-2 sample exceeded the acceptable limit on and after 9<sup>th</sup> day of the storage (Table 3). This higher TBARS value may be the results of a higher rate of secondary lipid oxidation due to the presence of high O<sub>2</sub> concentration in MAP-2 (50% CO<sub>2</sub> and 50% O<sub>2</sub>) samples. A study conducted by Arashisar *et al.* (2004) revealed a similar type of phenomenon in rainbow trout fillets packaged with 30% O<sub>2</sub>. It might be possible that O<sub>2</sub> along with some bacterial enzymes, also participated in the oxidation process (Hernandez *et al.*, 2009). Unpleasant odour or taste are often evident due to secondary oxidation of lipids (Jezek and Buchtova, 2012). The substances produced during lipid oxidation may be responsible for the textural and organoleptic changes of fish fillets, particularly when they form covalent bonds with muscle proteins (Huss, 1995). Thiobarbituric acid (TBA) is a more suitable indicator of the degree of fish muscle oxidation than peroxide value since the interaction of malonaldehyde may occur with other components such as nucleosides, nucleic acids, proteins, and other aldehydes.

Based on the literature, freshwater fishes that are freshly caught (tilapia, rainbow trout, silver perch, and sea bass) have shown bacterial counts around log 2 - 6 CFU/g (Gelman *et al.*, 2001). In the present work, the initial total viable count (TVC) of

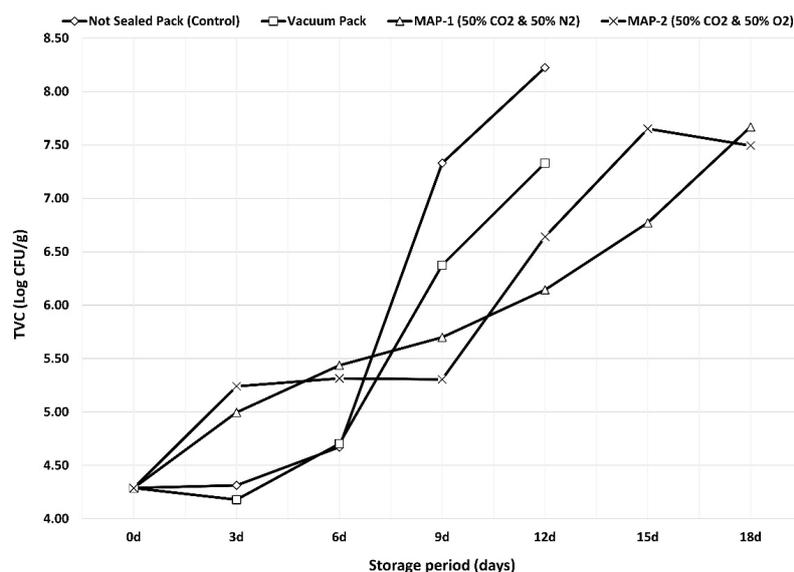


Figure 1. Changes in total viable count (TVC) of sliced Rohu fish under different packaging conditions at refrigerated storage (4°C).

sliced Rohu fish samples (log 4.29 CFU/g) indicated a satisfactory quality of fresh fish. Significantly ( $p < 0.05$ ) lower TVC values were recorded on 9<sup>th</sup> and 12<sup>th</sup> day of storage in all packaged samples as compared to control (Table 4). Babic *et al.* (2015) observed similar trend on common carp steaks kept under MAP (40% CO<sub>2</sub> and 60% N<sub>2</sub> and 100% CO<sub>2</sub>) followed by storage at  $3 \pm 0.5^\circ\text{C}$ . This result is also supported by Hudecova *et al.* (2010) during their study on fresh common carp at two different modified atmosphere packaging (MAP-1: 70% N<sub>2</sub> and 30% CO<sub>2</sub> and MAP-2: 80% O<sub>2</sub> and 20% CO<sub>2</sub>). They found that MAP-1 and MAP-2 showed better performance as compared to control at  $4 \pm 0.5^\circ\text{C}$ . In the present work, higher shelf life was observed in both MAPs, perhaps due to the bacteriostatic effect of CO<sub>2</sub> in the MAP. The impact of various CO<sub>2</sub> concentrations showed delayed microbial growth as concluded by multiple researchers such as in rainbow trout (Arashisar *et al.*, 2004), swordfish (Pantazi *et al.*, 2008), chub mackerel (Stamatis and Arkoudelos, 2007), and salmon (De la Hoz *et al.*, 2000).

According to the International Commission on Microbiological Specifications for Foods (ICMSF, 1986), the upper acceptable limit of aerobic plate counts (APC) for frozen and fresh fish is log 7 CFU/g. In the present work, the TVC exceeded the acceptable limit on approximately 8<sup>th</sup> day for control, 11<sup>th</sup> day for vacuum pack, 16<sup>th</sup> day for MAP-1, and 13<sup>th</sup> day for MAP-2 (Figure 1). Therefore, considering the TVC, the shelf life of sliced Rohu fish samples at refrigerated storage was determined at around 8, 11, 16, and 13 days for control, vacuum pack, MAP-1 (50% CO<sub>2</sub> and 50% N<sub>2</sub>), and

MAP-2 (50% CO<sub>2</sub> and 50% O<sub>2</sub>) sample, respectively. In the present work, MAP-1 (50% CO<sub>2</sub> and 50% N<sub>2</sub>) showed the best result in terms of shelf life with having a risk of growing harmful anaerobic bacteria. On the other hand, MAP-2 (50% CO<sub>2</sub> and 50% O<sub>2</sub>) presented lower shelf life than MAP-1, but has a low risk of harmful aerobic bacterial growth.

## Conclusion

Vacuum packaging, modified atmosphere packaging, and refrigerated storage effectively preserved fish and other value-added products. It can be concluded that all packaging systems provided satisfactory results during the storage period, except for the control which showed total bacterial counts above acceptable levels on the 7<sup>th</sup> day of storage. Vacuum packaging reduced the TVC and TBARS during the storage period, and extended the shelf life for 11 days. MAP-1 (50% CO<sub>2</sub> and 50% N<sub>2</sub>) reduced the TVC, TVB-N, and TBARS during the storage period, and extended shelf life for 16 days. MAP-2 (50% CO<sub>2</sub> and 50% O<sub>2</sub>) reduced the TVC and TVB-N during the storage period, and increased the shelf life for 13 days. Although MAP-1 yielded the highest shelf life to sliced Rohu fish, there is a concern with the growth of pathogenic anaerobic bacteria. MAP-2 had a lower shelf life than MAP-1, but there is less risk of developing these pathogenic bacteria that may be a concern for food security. Therefore, either MAP-2 or MAP-1 with additional control measures against anaerobic bacteria can be used by processors or superstores to display the fish and fishery products under refrigerated temperature

that will increase the product's shelf life, value, and convenience.

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