



## Impact of Modified Atmosphere Packaging on Shelf Life of Ready-to-Cook Tilapia (*Oreochromis niloticus*) Fish Balls

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### ABSTRACT

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The effects of modified atmosphere packaging (MAP) on the shelf life of tilapia (*Oreochromis niloticus*) fish balls during refrigerated storage (4°C) were investigated for 20 days. The moisture, protein, lipid, and carbohydrate contents of the fish balls were 61.60%, 13.61%, 6.62%, and 14.13%, respectively. The pH, total volatile base nitrogen (TVB-N), and thiobarbituric acid reactive substances (TBARS) of the tilapia fish ball samples during storage under control and MAP packaging were within acceptable limits. On the other hand, the aerobic plate count (APC) of tilapia fish balls gradually increased from the initial value of 4.37±0.04 on day 0 to 7.91±0.45 log CFU/g on 20th day for the not sealed pack (control). Significantly lower APC values (p<0.05) were observed on the 16th and 20th days in MAP-3 packaging compared with other packaging methods. The APCs exceeded 7 log CFU/g, which is considered the upper acceptable limit for fresh and frozen fish, on approximately the 14th day for the control, the 18th day for MAP-1, the 17th day for MAP-2, and the 20th day for MAP-3, respectively. Based on bacterial counts, the shelf life was determined to be 14 days for the control, 18 days for MAP-1, 17 days for MAP-2, and 20 days for MAP-3, respectively. Therefore, MAP is a good option for increasing the shelf life of fishery products, which supermarkets can adopt to display their products with an extended shelf life.

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## 1. Introduction

The preference for food choices has been changing for many people, particularly those who are doing a full-time job or business, along with their economic and social development. This ongoing current trend created a high demand for value-added fish products and ready-to-cook (RTC) meals (Rathod and Pagarkar 2013). Nowadays, many city dwellers, particularly working mothers and homemakers, crave RTC over raw ingredients in order to reduce the long cooking time (Adetunji and Odetokun 2012). RTC snack products have become popular and accepted by widespread people as the advancement of technologies. One of the advantages is that these products are easy to cook and don't require additional preparation and time; moreover, modern packaging is

appealing to many consumers. Value addition is a process that alters the characteristics of raw materials to increase a product's or service's value and is termed as value-added products. (Viji et al. 2015). For example, value-added fish products such as fish balls, fish crackers, fish marinades, fish sticks, fish fingers, and fish burgers are well known for their characteristic taste and flavor (Thi et al. 2013). Past studies revealed that fish can be utilized in Bangladesh to ensure food security through value-added fish products made from various fish mince (Hoque et al. 2021). There are limited value added fishery products available, and most are found primarily in Bangladeshi supermarkets.

Several enterprises in the nation have introduced frozen value-added meat products to the market (Amin et al.

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2024). The availability of value-added fish products in Bangladesh is still limited. However, consumer interest in these types of food products was driven by their appeal, reasonable prices, taste, appearance, and texture (Patel and Rathod 2017).

Fish balls are one of those RTC fishery products that have recently become widely recognized as a well-known value-added product. It is a low-cost product due to easy manufacturing as well as high nutritional value (Kok et al. 2013). It has numerous culinary applications and is also popular in Taiwan and other Asian nations. Fish meat, which is often heavy in protein and moisture, is the primary ingredient in fish balls, making them extremely perishable (Kok and Park 2007). At room temperature, the quality of fish balls remains for only 24 hours. According to Rohimadilwa et al. (2021), fish meat-based meatballs contain a pH of 6–6.5, which is nearly neutral, and possess high protein and water content. As a result, the shelf life at room temperature only last for one day. Perhaps, preservatives, combined with appropriate packaging and storage, can help extend the shelf life of meatballs (Meng et al. 2022).

Fish and fishery products undergo various physical and chemical changes during storage. These changes have a detrimental effect on the stability and quality of refrigerated fish products (Gram and Huss 1996). It is reported that due to inadequate packaging and storage conditions, a significant percentage of food products are contaminated with pathogens and become degraded (Karanth et al. 2023).

According to Tørngrena et al. (2018), the role of food packaging has evolved from basic, passive preservation methods to more sophisticated, active systems that extend beyond conventional applications. Some retailers have begun offering fish products packaged in polyethylene (PE) pouches as a standard practice; however, this approach does not necessarily ensure product quality preservation or shelf-life extension. In response to increasing consumer demand and the need to maintain product consistency and quality, the food packaging industry is adopting innovative packaging technologies. In this context, modified atmosphere packaging (MAP) has gained considerable attention.

Modified atmosphere packaging (MAP) is a preservation technique that alters the composition of the surrounding air of perishable foods by using one or a combination of protective gases (Arashisar et al. 2004). Alongside refrigerated storage, modified atmosphere packaging (MAP) has gained increasing popularity as a preservation technique for extending the shelf life of fresh meat and fish (Narasimha Rao and Sachindra, 2002). Various gases, including nitrogen (N<sub>2</sub>), carbon dioxide (CO<sub>2</sub>), oxygen (O<sub>2</sub>), and carbon monoxide (CO), are used in MAP applications. Among these, CO<sub>2</sub> is widely regarded as one of the most effective and commonly used gases due to its ability to dissolve in water and lipids (DeWitt and Oliveira, 2016). It plays a critical role as a bacteriostatic agent by inhibiting microbial growth, particularly during the logarithmic phase (Alfaro et al., 2013). However, its effectiveness depends on factors such as product type,

storage temperature, initial microbial load, and CO<sub>2</sub> concentration (DeWitt and Oliveira, 2016).

Oxygen (O<sub>2</sub>) is incorporated into MAP systems to inhibit the growth and toxin production of *Clostridium botulinum* type E, an obligate anaerobic bacterium (Pantazi et al., 2008). Additionally, it helps maintain the desirable colour of fish and meat products (Soccol and Oetterer, 2003). Nitrogen (N<sub>2</sub>), an inert gas with low solubility, is primarily used as a filler gas to displace oxygen, thereby reducing oxidative rancidity and inhibiting the growth of aerobic microorganisms. It also helps prevent package collapse caused by CO<sub>2</sub> absorption and can limit mould growth and insect infestation.

In developed countries, MAP has been widely adopted as an effective packaging technique, extending the shelf life of meat, fish, and other perishable products by approximately 25–100% (DeWitt and Oliveira, 2016). The effectiveness of MAP is influenced by several factors, including storage temperature, raw material quality, gas composition, and packaging materials. Overall, MAP contributes to reduced economic losses, enhanced product shelf life, and improved distribution potential, allowing products to reach distant markets and thereby increasing their commercial value (Pastoriza et al., 1996).

Although studies have been conducted on the shelf life of fish balls under different storage conditions but fish balls stored in MAP conditions have not been studied yet. Therefore, the objective of the present study was to develop and evaluate the quality of fish balls packaged using MAP in comparison with non-sealed packaging.

## 2. Materials and Methods

### 2.1. Sample collection

Tilapia (*Oreochromis niloticus*) fish with an average weight of 0.9±0.1 kg were purchased from the local market and transported in live condition to the Quality Control Laboratory, Department of Fisheries, University of Rajshahi.

### 2.2. Preparation of fish ball

Upon arrival, the fish were washed, skinned, and cut into pieces for mincing using a commercial meat mincer (MK-G1800P, Panasonic Corporation, Japan). Firstly, 500 g of fish mince was taken in a dough maker bowl, and then different ingredients (corn flour-40 g, ginger paste-10 g, garlic paste-8 g, coriander-2.5 g, cumin powder-2.5 g, pepper powder-3 g, black pepper powder-1.5 g, soya sauce-1/2 tsp., lemon juice-2 tsp., salt-7.5 g, onion flakes-40 g, chili-4 pieces, bread crumbs-1/2 cup, egg-2 pieces) were incorporated slowly and mixed well in the dough maker (MR-1042A, Miyako, China). The mixture was then kept in a standard refrigerator for 30 minutes, and then the fish balls were made manually, weighing around 25-30 g each. Then the ball was dipped in liquid egg mix and rolled in breadcrumbs. Finally, the fish ball was ready for the next step of packaging and preservation.

### 2.3. Packaging and storage

Around 80 g of fish balls were packed into low gas and moisture permeable, multi-layered (PE/PA/PE) transparent plastic pouches with a thickness of 100  $\mu$ m. The following four packing methods were employed: (1) not sealed pack as a control; (2) Treatment 1 is MAP-1 (50% CO<sub>2</sub> & 50% N<sub>2</sub>); Treatment 2 is MAP-2 (40% CO<sub>2</sub>, 30% N<sub>2</sub>, & 30% O<sub>2</sub>); and Treatment 3 is MAP-3 (50% CO<sub>2</sub> & 50% O<sub>2</sub>). MAP packaging was performed using a packaging machine (C100 Multivac, Germany) equipped with a gas mixer (KM100-3 MEM, WITT, Germany) and N<sub>2</sub>, O<sub>2</sub>, and CO<sub>2</sub> gas cylinders by following the manufacturer's instructions. Gas in the packed sample headspace was measured using a gas analyzer (Oxybaby M+, WITT, Germany). All packaged samples were kept in a lab refrigerator at 4°C. Three samples from each packaging condition were analyzed in the laboratory during 0, 4, 8, 16, and 20 days of storage.

### 2.4. Proximate composition

After preparing fish balls, proximate analysis (moisture, lipid, crude protein, and ash) was carried out using the AOAC method (AOAC 1980) with slight modifications. Carbohydrate content was calculated indirectly by subtracting the total amounts of protein, lipid, ash, and moisture from 100.

### 2.5. Biochemical and microbiological analyses

According to Binsi et al. (2015), the pH of the fish ball homogenate was measured with a pH meter (HI2002-Edge, Hanna Inst., USA). In this instance, the homogenate was created by mixing 50 mL of distilled water with 10 g of fish ball. Total volatile base nitrogen (TVB-N) was determined for a 10 g ground fish ball sample using perchloric acid, following the EC (2005) method. Thiobarbituric acid reactive substances (TBARS) values were determined using a spectrophotometer (1601PC,

Shimadzu, Japan) in accordance with Park et al. (2007). TBARS values were calculated as follows: TBARS value (mg malonaldehyde/kg) = optical density (O.D.)  $\times$  5.2. Aerobic plate count (APC) was determined using the standard plate count method on plate count agar, following the FDA method (Maturin and Peeler 2001). Plates were incubated at 35°C for 48 h, and then colonies were counted.

### 2.6. Statistical analysis

Each experiment was conducted in triplicate, and results are presented as the mean  $\pm$  standard deviation. For the shelf-life study, statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test in SPSS version 20, with significance set at  $p < 0.05$ .

## 3. Results

### 3.1. Proximate composition

Proximate composition is essential to evaluate the nutritional quality of a product. The Tilapia fish balls produced for this study have a protein content of 13.61 $\pm$ 0.64%. The moisture, ash, lipid, and carbohydrate contents were 61.60 $\pm$ 0.57%, 4.35 $\pm$ 0.32%, 6.62 $\pm$ 1.01%, and 14.13 $\pm$ 0.28%, respectively. The estimated calorie content of the fish ball was 170.5 kcal per 100 g serving.

### 3.2. pH value

The initial pH of the Tilapia ball was 6.34. Following that, the pH readings displayed a decreasing trend with slight variations across all packaging settings. The pH of the MAP samples was significantly lower ( $p < 0.05$ ) than the control sample during the 4th, 16th, and 20th days of storage. However, on the 8th day of storage, the pH value was significantly higher ( $p < 0.05$ ) in MAP-3 samples compared to other samples (Table 1).

**Table 1.** Changes in the pH value of Tilapia fish ball under modified atmosphere packaging at 4°C

| Treatments   | Storage period (days)        |                               |                               |                              |                               |                              |
|--|------------------------------|-------------------------------|-------------------------------|------------------------------|-------------------------------|------------------------------|
|  | 0                            | 4                             | 8                             | 12                           | 16                            | 20                           |
| Not sealed pack (control)  | 6.34 $\pm$ 0.06 <sup>a</sup> | 6.31 $\pm$ 0.06 <sup>b</sup>  | 6.01 $\pm$ 0.11 <sup>a</sup>  | 5.92 $\pm$ 0.54 <sup>a</sup> | 6.01 $\pm$ 0.12 <sup>b</sup>  | 6.11 $\pm$ 0.02 <sup>b</sup> |
| MAP-1 (50% CO <sub>2</sub> & 50% N <sub>2</sub> )                      | 6.34 $\pm$ 0.06 <sup>a</sup> | 5.86 $\pm$ 0.23 <sup>a</sup>  | 6.06 $\pm$ 0.02 <sup>ab</sup> | 5.79 $\pm$ 0.31 <sup>a</sup> | 5.65 $\pm$ 0.22 <sup>ab</sup> | 5.51 $\pm$ 0.38 <sup>a</sup> |
| MAP-2 (40% CO <sub>2</sub> , 30% N <sub>2</sub> & 30% O <sub>2</sub> ) | 6.34 $\pm$ 0.06 <sup>a</sup> | 5.94 $\pm$ 0.03 <sup>a</sup>  | 6.10 $\pm$ 0.10 <sup>ab</sup> | 6.13 $\pm$ 0.16 <sup>a</sup> | 5.43 $\pm$ 0.16 <sup>a</sup>  | 5.23 $\pm$ 0.05 <sup>a</sup> |
| MAP-3 (50% CO <sub>2</sub> & 50% O <sub>2</sub> )                      | 6.34 $\pm$ 0.06 <sup>a</sup> | 6.03 $\pm$ 0.04 <sup>ab</sup> | 6.24 $\pm$ 0.02 <sup>b</sup>  | 6.26 $\pm$ 0.06 <sup>a</sup> | 5.64 $\pm$ 0.12 <sup>ab</sup> | 5.02 $\pm$ 0.15 <sup>a</sup> |

Different superscripts in the same column represent a significant difference between the means of treatments ( $p < 0.05$ )

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

In the present study, the initial TVB-N value of the Tilapia fish ball was 1.96 mg/100 g. The values were gradually decreased until the 8th day for the control, MAP-1 & MAP-2 samples, and until the 12th day for the MAP-3 samples; thereafter, the values increased with some fluctuations

under all packaging conditions (Table 2). Compared with the control, lower TVB-N values were observed in all other samples from day 12 onwards. However, no significant differences in TVB-N values were detected during storage ( $p > 0.05$ ).

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

The initial TBARS value in Tilapia fish balls was 0.94 mg MDA/kg. TBARS values fluctuated during the storage

period but exhibited an overall increasing trend over time (Table 3). However, TBARS values were significantly lower ( $p < 0.05$ ) in MAP-1 samples from day 4 onward compared to the control, MAP-2, and MAP-3 samples (Table 3). On most storage days, MAP-3 samples showed relatively higher TBARS values than MAP-1 and MAP-2.

**Table 2.** Changes in total volatile basic nitrogen (TVB-N) values (mg/100g) of Tilapia fish ball under modified atmosphere packaging at 4°C

| Treatments   | Storage period (days)  |                        |                        |                        |                        |                        |
|--|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|
|  | 0                      | 4                      | 8                      | 12                     | 16                     | 20                     |
| Not sealed pack (control)  | 1.96±0.79 <sup>a</sup> | 2.38±0.20 <sup>a</sup> | 1.82±0.20 <sup>a</sup> | 1.96±0.40 <sup>a</sup> | 2.24±0.40 <sup>a</sup> | -                      |
| MAP-1 (50% CO <sub>2</sub> & 50% N <sub>2</sub> )                      | 1.96±0.79 <sup>a</sup> | 1.82±0.20 <sup>a</sup> | 1.40±0.79 <sup>a</sup> | 1.54±0.20 <sup>a</sup> | 1.54±0.20 <sup>a</sup> | 2.66±0.20 <sup>a</sup> |
| MAP-2 (40% CO <sub>2</sub> , 30% N <sub>2</sub> & 30% O <sub>2</sub> ) | 1.96±0.79 <sup>a</sup> | 1.68±0.40 <sup>a</sup> | 1.26±0.20 <sup>a</sup> | 1.40±0.40 <sup>a</sup> | 1.68±0.40 <sup>a</sup> | 2.52±0.79 <sup>a</sup> |
| MAP-3 (50% CO <sub>2</sub> & 50% O <sub>2</sub> )                      | 1.96±0.79 <sup>a</sup> | 1.96±0.40 <sup>a</sup> | 2.10±0.59 <sup>a</sup> | 1.54±0.59 <sup>a</sup> | 1.82±0.20 <sup>a</sup> | 2.52±0.40 <sup>a</sup> |

Different superscripts in the same column represent a significant difference between the means of treatments ( $p < 0.05$ )

**Table 3.** Changes in thiobarbituric acid reactive substances (TBARS) values (mg MDA/kg fish) of Tilapia fish ball under modified atmosphere packaging at 4°C

| Treatments   | Storage period (days)  |                        |                        |                        |                        |           |
|--|------------------------|------------------------|------------------------|------------------------|------------------------|-----------|
|  | 0                      | 4                      | 8                      | 12                     | 16                     | 20        |
| Not sealed pack (control)  | 0.94±0.21 <sup>a</sup> | 0.74±0.15 <sup>b</sup> | 1.17±0.11 <sup>b</sup> | 1.07±0.28 <sup>a</sup> | 1.18±0.03 <sup>c</sup> |           |
| MAP-1 (50% CO <sub>2</sub> & 50% N <sub>2</sub> )                      | 0.94±0.21 <sup>a</sup> | 0.40±0.01 <sup>a</sup> | 0.94±0.02 <sup>a</sup> | 0.94±0.04 <sup>a</sup> | 0.72±0.02 <sup>a</sup> | 1.58±0.29 |
| MAP-2 (40% CO <sub>2</sub> , 30% N <sub>2</sub> & 30% O <sub>2</sub> ) | 0.94±0.21 <sup>a</sup> | 0.42±0.02 <sup>a</sup> | 1.00±0.03 <sup>a</sup> | 1.42±0.06 <sup>b</sup> | 1.03±0.10 <sup>b</sup> | 1.45±0.11 |
| MAP-3 (50% CO <sub>2</sub> & 50% O <sub>2</sub> )                      | 0.94±0.21 <sup>a</sup> | 0.71±0.03 <sup>b</sup> | 0.96±0.13 <sup>a</sup> | 1.42±0.09 <sup>b</sup> | 1.46±0.07 <sup>d</sup> | 1.59±0.23 |

Different superscripts in the same column represent a significant difference between the means of treatments ( $p < 0.05$ )

### 3.5. Aerobic plate count (APC)

Tilapia fish balls exhibited acceptable initial quality, with an APC of 4.37 log CFU/g. Throughout the storage period, APC values increased progressively under all packaging conditions. No significant differences ( $p > 0.05$ ) in APC

were observed among the treated samples up to 12 days of storage (Table 4). However, on days 16 and 20, APC values in MAP-3 samples were significantly lower ( $p < 0.05$ ) than those in the control and other treated samples.

**Table 4.** Changes in aerobic plate count (APC; log CFU/g) of Tilapia fish ball under modified atmosphere packaging at 4°C

| Treatments  | Storage period (days)  |                        |                        |                        |                        |                         |
|---|------------------------|------------------------|------------------------|------------------------|------------------------|-------------------------|
|   | 0                      | 4                      | 8                      | 12                     | 16                     | 20                      |
| Not sealed pack (control)   | 4.37±0.04 <sup>a</sup> | 4.52±0.12 <sup>a</sup> | 4.83±0.11 <sup>a</sup> | 6.02±0.25 <sup>a</sup> | 7.54±0.07 <sup>c</sup> | 7.91±0.45 <sup>b</sup>  |
| MAP-1 (50% CO <sub>2</sub> & 50% N <sub>2</sub> )                     | 4.37±0.04 <sup>a</sup> | 4.67±0.02 <sup>a</sup> | 5.93±0.01 <sup>a</sup> | 5.96±0.28 <sup>a</sup> | 6.76±0.03 <sup>b</sup> | 7.22±0.24 <sup>ab</sup> |
| MAP-2 (40%CO <sub>2</sub> , 30% N <sub>2</sub> & 30% O <sub>2</sub> ) | 4.37±0.04 <sup>a</sup> | 4.72±0.12 <sup>a</sup> | 5.30±0.87 <sup>a</sup> | 6.29±0.03 <sup>a</sup> | 6.93±0.30 <sup>b</sup> | 7.17±0.09 <sup>ab</sup> |
| MAP-3 (50% CO <sub>2</sub> & 50% O <sub>2</sub> )                     | 4.37±0.04 <sup>a</sup> | 4.68±0.10 <sup>a</sup> | 5.79±0.03 <sup>a</sup> | 6.01±0.21 <sup>a</sup> | 6.15±0.20 <sup>a</sup> | 7.06±0.06 <sup>a</sup>  |

Different superscripts in the same column represent a significant difference between the means of treatments ( $p < 0.05$ )

## 4. Discussion

Proximate composition is an important parameter to evaluate the nutritional quality of a product. Tilapia fish balls produced in this study contained 13.61% protein. This finding is generally consistent with the results

reported by Huda et al. (2000), who observed protein contents ranging from 8.3% to 14.6%. The moisture content was 61.60%, which is lower than the range (72.5–89.9%) reported for Malaysian fish balls in a study conducted by Huda et al. (2000). In contrast, the carbohydrate content of the present samples was 14.13%,

exceeding the previously reported range of 0.2–11.7%. The ash content was 4.35%, which is slightly higher than the values (1.1–2.7%) reported by Huda et al. (2000).

The pH of fish muscle is an important indicator of microbial activity (Basdeki et al. 2025). Certain proteolytic bacteria produce organic acids during carbohydrate degradation, thereby increasing the acidity of the fish. The post-mortem pH of fresh fish typically ranges from 6.8 to 7.0 (Metin et al., 2001), and the pH values of all samples in this study were within this acceptable range. Similar findings were reported by Balange et al. (2004), who observed a decrease in pH from 5.90 to 4.20 in chilled fish balls during storage. Generally, microorganisms preferentially metabolize carbohydrates before utilizing proteins, resulting in acid production and a subsequent decline in pH (Frazier and Westhoff, 1988).

In modified atmosphere packaging (MAP) systems containing CO<sub>2</sub>, the decline in pH may also be attributed to the dissolution of CO<sub>2</sub> in muscle tissue (Ježek and Buchtová, 2012). Absorbed CO<sub>2</sub> reacts with water to form carbonic acid, further contributing to pH reduction (Banks et al., 1980). However, an opposite trend has also been reported; Milijašević et al. (2017) observed a slight increase in pH after five days of storage in carp cuts packaged under MAP (80% O<sub>2</sub> + 20% CO<sub>2</sub>), which was attributed to the accumulation of basic compounds produced by spoilage bacteria. Total volatile basic nitrogen (TVB-N), which comprises ammonia (NH<sub>3</sub>), dimethylamine (DMA), and trimethylamine (TMA), is a key indicator of seafood freshness and quality (Wu and Bechtel, 2008). According to EU fish hygiene regulations, TVB-N is used as a chemical index when organoleptic assessment raises concerns about fish freshness (Castro et al., 2006).

A comparable trend was reported by Soccol et al. (2005), who observed no significant variation in TVB-N values among Tilapia samples packaged under modified atmosphere (60% CO<sub>2</sub>/40% O<sub>2</sub>) packaging during 20 days of storage at 1°C. Generally, TVB-N values increase with the progression of spoilage due to microbial activity, which degrades protein and non-protein nitrogenous compounds, leading to the accumulation of basic substances. This results in the formation of biogenic compounds such as ammonia, DMA, and TMA, which may contribute to an increase in pH during the later stages of storage (Goulas and Kontominas, 2007).

The acceptable limit for TVB-N in chilled fish is typically reported to range from 30 to 35 mg N/100 g (Connell, 1995). Studies have consistently shown that fish stored under modified atmosphere packaging (MAP) exhibit lower TVB-N levels compared to air-packed samples. For instance, Fagan et al. (2004) reported reduced TVB-N levels in MAP-stored samples compared to mackerel fillets stored in air. The increase in TVB-N observed during extended storage can be attributed to microbial growth and endogenous enzymatic activity (Ruiz-Capillas and Moral, 2001; Islami et al., 2015).

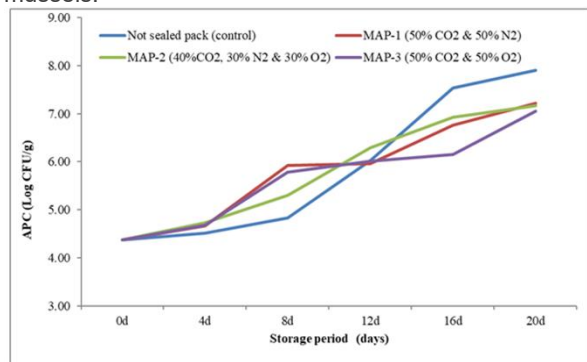
These findings are consistent with previous studies demonstrating a strong correlation between quality deterioration and increased TVB-N levels in MAP-treated seafood (Özogul et al., 2004; Nayma et al., 2020). Furthermore, Masniyom et al. (2002) reported lower TVB-N values in sea bass stored under higher CO<sub>2</sub> concentrations. In general, air-packed (control) samples tend to exhibit higher TVB-N values during storage compared to those stored in CO<sub>2</sub>-enriched atmospheres. Similar observations were reported by Ordóñez et al. (2000) for hake stored under MAP, supporting the results of the present study.

Thiobarbituric acid reactive substances (TBARS) are commonly used as a good indicator of lipid oxidation and overall food quality. However, Aubourg (1993) reported that TBARS measurements may not always accurately reflect the extent of lipid oxidation, as malondialdehyde can interact with proteins, amino acids, phospholipids, amines, nucleosides, and nucleic acids, as well as with other aldehydes formed during lipid oxidation. These interactions can vary considerably among various fish species. Furthermore, DeWitt and Oliveira (2016) reported that the effectiveness of thiobarbituric acid (TBA) values and TBARS as indicators of shelf life depends on the type and composition of the product.

In the absence of O<sub>2</sub>, modified atmosphere (MA)-packaged Tilapia and Goonch fish exhibited lower malondialdehyde (MDA) levels than the control samples (Masniyom et al., 2013; Alice et al., 2020). The acceptable limit for TBARS in fish is generally set at 2 mg MDA/kg, beyond which undesirable taste and odour may develop (Connell, 1995). In the present study, TBARS values remained below this threshold throughout the storage period for all samples (Table 3). Arashisar et al. (2004) also reported no significant changes in TBARS in Rainbow trout fillets stored under 100% CO<sub>2</sub> for up to 14 days. However, Masniyom et al. (2000) observed an increase in TBARS value in Sea Bass during storage at both MAP and air-packed samples, indicated that lipid oxidation might still occur even under high CO<sub>2</sub> conditions.

Aerobic plate count (APC) is a critical parameter for evaluating shelf life and microbiological quality. The APC of the ready-to-cook (RTC) Tilapia fish balls in the present study was 4.37 log CFU/g, indicating acceptable initial quality. For freshly harvested freshwater fish such as Silver Perch, Sea Bass, Rainbow Trout, and Tilapia, bacterial loads of 2-6 log CFU/g are considered acceptable (Gelman et al., 2001). The International Commission on Microbiological Specifications for Foods (ICMSF, 1986) recommends a maximum limit of 7 log CFU/g for APC in ready-to-cook fishery products. In the present study, APC values exceeded this limit approximately on day 14 for the control, day 18 for MAP-1, day 17 for MAP-2, and day 20 for MAP-3 (Figure 1). Similarly, Nosedá et al. (2012) reported extended shelf life in Vietnamese pangasius fillets stored under MAP conditions containing 50% CO<sub>2</sub> and 50% O<sub>2</sub>.

Microbiological evaluation by Reddy et al. (1995) showed that Tilapia fillets packaged under MA conditions (75% CO<sub>2</sub>/25% N<sub>2</sub>) and stored at 4°C had a shelf life exceeding 25 days, which is longer than observed in the present study. This difference may be attributed to the higher concentrations of CO<sub>2</sub> and N<sub>2</sub> used. Nitrogen flushing alone is generally insufficient to significantly extend shelf life. Goulas et al. (2005) demonstrated that higher CO<sub>2</sub> levels (up to 80%) effectively inhibit microbial growth in mussels.



**Figure 1.** Changes in aerobic plate count (log CFU/g) of Tilapia fish balls under modified atmosphere packaging during storage at 4°C.

While CO<sub>2</sub>-enriched packaging can successfully retard microbial spoilage, it does not completely prevent chemical deterioration, particularly lipid oxidation and associated physical changes. Masniyom et al. (2000) reported that sea bass slices packaged under 80–100% CO<sub>2</sub> had a shelf life of up to 20 days at 4°C, compared to only 9 days for air-packaged samples.

## 5. Conclusions

The findings of this study determined that modified atmosphere packaging (MAP), when combined with refrigerated storage, is an efficient strategy for extending the shelf life and preserving the quality of Tilapia fish balls by suppressing microbial growth and reducing oxidative deterioration compared to conventional air packaging. Evaluation of biochemical and microbiological parameters confirmed that MAP-treated samples maintained overall better quality throughout the storage, with MAP containing 50% CO<sub>2</sub> and 50% O<sub>2</sub> showing the most pronounced inhibitory effect on bacterial proliferation and consequently providing the longest shelf life. Although the presence of oxygen in this gas composition contributed to a relatively higher rate of lipid oxidation, TBARS values remained within acceptable limits over the entire storage period, indicating that oxidative changes did not adversely affect product quality. Furthermore, the inclusion of oxygen plays a critical role in limiting the growth of anaerobic pathogens such as *Clostridium* spp., thereby enhancing product safety. Despite these advantages, the wider adoption of MAP technology may be constrained by factors such as higher processing and equipment costs, the requirement for skilled personnel, and the need to ensure high initial raw material quality to minimize contamination. Nevertheless, MAP presents a promising

and practical approach for improving the safety, quality, and marketability of fish and fishery products, and its execution in commercial retail settings, such as superstores, could significantly benefit both producers and consumers.

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## References

- Adetunji VO, Odetokun IA. 2012. Fish species availability observed at fish landing centres in Bangladesh. *Research Journal of Microbiology*, 7:235-241. <https://doi.org/10.1088/1755-1315/414/1/012015>
- Alfaro B, Hernández I, Le Marc Y, Pin C. 2013. Modeling the effect of the temperature and carbon dioxide on the growth of spoilage bacteria in packed fish products. *Food Control*, 29(2):429-437. doi: 10.1016/j.foodcont.2012.05.046
- Alice EJ, Amanullah M, Karim MA, Hossain MA, Islam MT. 2020. Effects of vacuum and modified atmosphere packaging on the biochemical and microbiological quality of sliced gizzard fish (*Bagarius bagarius*) stored at refrigerated conditions. *Food Research*, 4(6): 2256-2264. [http://dx.doi.org/10.26656/fr.2017.4\(6\).287](http://dx.doi.org/10.26656/fr.2017.4(6).287)
- Amin MR, Ali MS, Rahman KM, Sarker MS, Sharmin MF, Hashem MA. 2024. Market and value chain of chicken meat products in Bangladesh: A revolutionary impact on employment generation, enterprise development and food security. *Meat Research*, 4(4):1-11. <https://doi.org/10.55002/mr.4.4.97>
- AOAC (Association of Official Analytical Chemists). (1980). In: Horwitz N (ed). *Official methods of analysis*, Association of Official Analytical Chemists, 13<sup>th</sup> ed., Washington, D., p. 957.
- Arashisar S, Hisar O, Kaya M, Yanik T. 2004. Effects of modified atmosphere and vacuum packaging on microbiological and chemical properties of rainbow trout (*Oncorhynchus mykiss*) fillets. *International Journal of Food Microbiology*, 97(2):209-214. <https://doi.org/10.1016/j.ijfoodmicro.2004.05.024>
- Aubourg SP. 1993. Review: Interaction of malondialdehyde with biological molecules —new trends in reactivity and significance. *International Journal of Food Science and Technology*, 28:323-335. <https://doi.org/10.1111/j.1365-2621.1993.tb01278.x>
- Balange AK, Joshi VR, Pagarkar AU. 2004. Preparation of pasteurized fish ball in curry and its storage study. *Journal of Indian Fisheries Association*, 30: 141-148.
- Banks H, Ranzell N, Finne G. 1980. Shelf-life studies on carbon dioxide finfish from the Gulf of Mexico. *Journal of Food Science*, 45(2):157-162. <http://dx.doi.org/10.1111/j.1365-2621.1980.tb02566.x>
- Basdeki E, Vasilaki SE, Sensi M, Fletmetakis E, Biscarini F, Power D, Tsironi T. 2025. Reviewing the correlation of fish quality alteration and in-package headspace composition: evidence from a pH freshness indicator case study. *International Journal of Food Science*, 2025(1):3576183. <https://doi.org/10.1155/ijfo/3576183>
- Binsi PK, Viji P, Visnuvinayagam S, Ninan G, Sangeeta G, Triveni A, Ravishankar CN. 2015. Microbiological and shelf-life characteristics of eviscerated and vacuum packed freshwater catfish (*Ompok pabda*) during chill storage. *Journal of Food Science and Technology*, 52(3):1424-1433. <https://doi.org/10.1007/s13197-013-1165-x>
- Castro P, Padro JCP, Cansino MJ. 2006. Total volatile base nitrogen and its use to assess freshness in European sea bass stored in ice. *Food Control*, 17: 245-248.
- Connell JJ. 1990. *Control of fish quality*. 3<sup>rd</sup> ed. Fishing News Books, Oxford, UK.
- Connell JJ. 1995. *Control of fish quality*. 4<sup>th</sup> ed. Wiley-Blackwell, Oxford, UK.
- DeWitt CAM, Oliveira A. 2016. Modified atmosphere systems and shelf-life extension of fish and fishery products. *Foods*, 5(3):48.

- <https://doi.org/10.3390/foods5030048>
- European Commission (EC). 2005. Determination of the concentration of TVB-N in fish and fishery products. Commission Regulation, Chapter III. (EC) No 2074/2005. Official Journal of European Union, L37: 338.
- Fagan JD, Gormley TR, Mhuirheartaigh, MU. 2004. Effect of modified atmosphere packaging with freeze-chilling on some quality parameters of raw whiting, mackerel and salmon portions. *Innovative Food Science & Emerging Technologies*, 5(2):205-214.
- Frazier WC, Westhoff DC. 1988. *Food Microbiology*, Tata McGraw-Hill Publishing Company Limited, Uttar Pradesh, India.
- Gelman A, Glatman L, Drabkin V, Harpaz S. 2001. Effects of storage temperature and preservative treatment on shelf-life of the pond-raised freshwater fish, silver perch (*Bidyanus bidyanus*). *Journal of Food Protection*, 64(10):1584-1591. <https://doi.org/10.4315/0362-028X-64.10.1584>
- Goulas AE, Chouliara I, Nessi E, Kontominas MG, Savvaidis IN. 2005. Microbiological, biochemical and sensory assessment of mussels (*Mytilus galloprovincialis*) stored under modified atmosphere packaging. *Journal of Applied Microbiology*, 98:752-760.
- Goulas AE, Kontominas MG. 2007. Combined effect of light salting, modified atmosphere packaging and oregano essential oil on the shelf-life of sea bream (*Sparus aurata*): Biochemical and sensory attributes. *Food Chemistry*, 100:287-296. <https://doi.org/10.1016/j.foodchem.2005.09.045>
- Gram L, Huss HH. 1996. Microbiological spoilage of fish and fish products. *International Journal of Food Microbiology*, 33(1):121-37.
- Hoque MS, Roy S, Mukit SS, Rahman MB, Akter S. 2021. Effects of Pangasius (*Pangasius hypophthalmus*) and Skipjack Tuna (*Sarda orientalis*) mince blend on the quality of fish products: Ways to utilize resources and nutrition in Bangladesh. *Food Science & Nutrition*, 9(12):6642-6652. <https://doi.org/10.1002/fsn3.2612>
- Huda N, Aminah A, Babji AS. 2000. Physicochemical properties of Malaysian fish balls. *Fishery Technology*, 38: 14-17.
- International Commission on Microbiological Specifications for Foods (ICMSF). 1986. *Microorganisms in Food-2, Sampling for Microbiological Analysis: Principles and Specific Applications*. 2<sup>nd</sup> ed. University of Toronto Press, Toronto, Canada.
- Islami SNE, Faisal M, Akter M, Reza MS, Kamal M. 2015. Comparative shelf-life study of whole fish and fillets of cultured striped catfish (*Pangasianodon hypophthalmus*) during ice storage condition. *Research of Agricultural Livestock and Fisheries*, 2(1):177-183. <http://dx.doi.org/10.3329/ralf.v2i1.23056>
- Jezek F, Buchtova H. 2012. Effect of modified atmosphere packaging on the course of physical and chemical changes in chilled muscle tissue of silver carp (*Hypophthalmichthys molitrix*, V.). *Polish Journal of Veterinary Sciences*, 15(3):439-445. <https://doi.org/10.2478/v10181-012-0068-5>
- Karant S, Feng S, Patra D, Pradhan AK. 2023. Linking microbial contamination to food spoilage and food waste: The role of smart packaging, spoilage risk assessments, and date labeling. *Frontiers in Microbiology*, 14:1198124. <https://doi.org/10.3389/fmicb.2023.1198124>
- Kok N, Thawornchinsombut S, Park JW. 2013. *Manufacture of Fish Balls*. In: Park JW (ed) *Surimi and surimi seafood* 3<sup>rd</sup> eds. CRC Press, Boca Raton, USA. pp.666. <https://doi.org/10.1201/b16009>
- Kok TN, Park JW. 2007. Extending the shelf life of set fish ball. *Journal of Food Quality*, 30(1): 1-27.
- Masniyom P, Benjakul S, Visessanguan W. 2002. Shelf-life extension of refrigerated seabass slices under modified atmosphere packaging. *Journal of the Science of Food and Agriculture*, 82(8):873-880.
- Masniyom P, Benjama O, Maneesri J. 2013. Effect of modified atmosphere and vacuum packaging on quality changes of refrigerated tilapia (*Oreochromis niloticus*) fillets. *International Food Research Journal*, 20(3):1401-1408.
- Maturin, L, Peeler, JT. 2001. *BAM: Aerobic plate count*. US Food and Drug Administration: Silver Spring, MD, USA, 1-10.
- Meng X, Wu D, Zhang Z, Wang H, Wu P, Xu Z, Gao Z, Mintah BK, Dabbour M. 2022. An overview of factors affecting the quality of beef meatballs: Processing and preservation. *Food Science & Nutrition*, 10(6):1961-74. <https://doi.org/10.1002/fsn3.2812>
- Metin S, Erkan N, Variik C, Aran N. 2001. Extension of shelf-life of chub mackerel (*Scomber japonicus* Houttuyn 1780) treated with lactic acid. *European Food Research and Technology*, 213:174-177. <http://dx.doi.org/10.1007/s002170100362>
- Milijašević M, Babić Milijašević J, Đinović-Stojanović J, Vesković Moračanin S, Lilić S. 2017. Changes of pH and peroxide value in carp (*Cyprinus carpio*) cuts packaged in modified atmosphere IOP Publishing, IOP Conf. Series: Earth and Environmental Science 85 012041
- Narasimha RD, Sachindra NM. 2002. Modified atmosphere and vacuum packaging of meat and poultry products. *Food Reviews International*, 18(4): 263-293. doi: 10.1081/FRI-120016206
- Nayma K, Das KC, Alice EJ, Mehbub MF, Islam MT. 2020. Extension of shelf-life of ready-to-cook (RTC) pangas fish (*Pangasianodon hypophthalmus*) curry by modified atmosphere packaging at chilled storage. *IOP Conf. Series: Earth and Environmental Science*, 414, 01201. <http://dx.doi.org/10.1088/1755-1315/414/1/012015>
- Noseda B, Islam MT, Eriksson M, Heyndrickx M, Reu KD, Langenhove HV, Devlieghere F. 2012. Microbiological spoilage of vacuum and modified atmosphere packaged Vietnamese *P. hypophthalmus* filets. *Food Microbiology*, 30(2): 408-419.
- Ordóñez JA, López-Gálvez DE, Fernández M, Hierro E, de la Hoz L. 2000. Microbial and physicochemical modifications of hake (*Merluccius merluccius*) steaks stored under carbon dioxide enriched atmospheres. *Journal of the Science of Food and Agriculture*, 80(13):1831-1840.
- Özogul F, Polat A, Ozogul Y. 2004. The effects of modified atmosphere packaging and vacuum packaging on chemical, sensory and microbiological changes of sardines (*Sardina pilchardus*). *Food Chemistry*, 85(1):49-57. <https://doi.org/10.1016/j.foodchem.2003.05.006>
- Pantazi D, Papavergou A, Pournis N, Kontominas MG, Savvaidis IN. 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. doi: 10.1016/j.fm.2007.06.006
- Park SY, Yoo SS, Uh JH, Eun JB, Lee HC, Kim YJ, Chin KB. 2007. Evaluation of lipid oxidation and oxidative products as affected by pork meat cut, packaging method, and storage time during frozen storage (-10°C). *Journal of Food Science*, 72(2): 114-119. <https://doi.org/10.1111/j.1750-3841.2006.00265.x>
- Pastoriza L, Sampedro G, Herrera JJ, Cabo ML. 1996. Effect of modified atmosphere packaging on shelf-life of iced fresh hake slices. *Journal of the Science of Food and Agriculture*, 71(4):541-547.
- Patel D, Rathod R. 2017. Ready-to-eat food perception, food preferences and food choice—a theoretical discussion. *Worldwide Journal of Multidisciplinary Research and Development*, 3(8):198-205.
- Rathod N, Pagarkar A. 2013. Biochemical and sensory quality changes of fish cutlets, made from Pangasius fish (*Pangasianodon hypophthalmus*), during storage in refrigerated display unit at -15 to -18°C. *International Journal of Food, Agriculture and Veterinary Science*, 3(1):1-8.
- Reddy NR, Villanueva M, Kautter DA. 1995. Shelf-life of modified-atmosphere-packaged fresh tilapia fillets stored under refrigeration and temperature-abuse conditions. *Journal of Food Protection*, 58:908-914. <https://doi.org/10.4315/0362-028X-58.8.908>
- Rohimadilwa I, Windyasmara L, Sukaryani S. 2021. Effect of chicken meatball shelf life with adding chitosan to chemical quality. *Bantara Journal of Animal Science*, 3(1): 9-15. <https://doi.org/10.32585/bjas.v3i1.1313>
- Ruiz-Capillas C, and Moral A. 2001. Chilled bulk storage of gutted hake (*Merluccius merluccius* L.) in CO<sub>2</sub> and O<sub>2</sub> enriched controlled atmospheres. *Food Chemistry*, 74:317-325. [https://doi.org/10.1016/S0308-8146\(01\)00159-5](https://doi.org/10.1016/S0308-8146(01)00159-5)
- Socol MCH, Oetterer M, Gallo CR, Spoto MF, Biato DO. 2005. Effects of modified atmosphere and vacuum on shelf life of tilapia (*Oreochromis niloticus*) fillets. *Brazilian Journal of Food Technology*, 8:7-15.
- Socol MCH, Oetterer M. 2003. Use of modified atmosphere in seafood preservation. *Brazilian Archives of Biology and Technology*, 46(4):569-580. <https://doi.org/10.1590/S1516-89132003000400011>

- Thi ANT, Nosedá B, Samapundo S, Nguyen B L, Broekaert K, Rasschaert G, Devlieghere F. 2013. Microbial ecology of Vietnamese Tra fish (*Pangasius hypophthalmus*) fillets during processing. International Journal of Food Microbiology, 167(2):144-152. <https://doi.org/10.1016/j.ijfoodmicro.2013.09.010>
- Tørngrena MA, Darréa M, Gunviga A, Bardenshtein A. 2018. Case studies of packaging and processing solutions to improve meat quality and safety. Meat Science, 144:149-158.
- Viji P, Tanuja S, Ninan G, Lalitha K, Zynudheen AA, Binsi PK, Srinivasagopal TK. 2015. Biochemical, textural, microbiological and sensory attributes of gutted and ungutted sutchi catfish (*Pangasianodon hypophthalmus*) stored in ice. Journal of Food Science and Technology, 52 (6): 3312-3321. <https://doi.org/10.1007/s13197-014-1358-y>
- Wu TH, Bechtel PJ. 2008. Ammonia, Dimethylamine, Trimethylamine, and Trimethylamine Oxide from raw and processed fish by-products. Journal of Aquatic Food Product Technology, 17(1):27-38. <https://doi.org/10.1080/10498850801891140>