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Research Article

Effects of Modified Atmosphere Packaging (MAP) on Quality and Shelf Life of Dried Spotted Snakehead (*Channa punctatus*) Products Using an Improved Solar Dryer

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ABSTRACT

This study focused on follow-up the changes in the quality and shelf life of dried spotted snakehead (*Channa punctatus*) products under modified atmosphere packaging (MAP) conditions processed using an improved solar dryer. Four packaging methods, such as, air pack (sealed without changing atmospheric gas), MAP-1 (100% N_2), MAP-2 (50% CO_2 & 50% N_2) and MAP-3 (40% CO_2, 30% O_2 & 30% N_2) were employed and kept at room temperature. Biochemical and microbial analyses were performed at 15-days intervals over a period of 4 to 5 months of room storage. Moisture of the dried products remained within acceptable limits considering the salt content of the products. Observations revealed comparatively lower pH, total volatile base nitrogen (TVB-N), and thiobarbituric acid reactive substance (TBARS) values, none of which crossed the acceptable limits in any of the packaging conditions. Total viable counts (TVC) exceeded the permissible level of 5 log $CFUg^{-1}$ after 75 days for air pack and MAP-1 samples and 120 days for MAP-2 and MAP-3 samples. Based on the bacterial counts, the shelf life of dried spotted snakehead products was estimated at approximately 75 days for air-pack and MAP-1 samples and 120 days for MAP-2 & MAP-3 samples. Therefore, MAP-2 (50% CO_2 & 50% N_2) & MAP-3 (40% CO_2, 30% O_2 & 30% N_2) were identified as effective packaging methods for extending the shelf life of dried spotted snakehead products.

Introduction

In Bangladesh, sun drying is considered one of the most important and widely practiced methods for fish preservation. It is a crucial source of income and sustenance for the poor and economically disadvantaged coastal populations (Nowsad, 2005). A large number of people, including women, are involved in this industry. About 20% of the artisanal fish caught are sun-dried and consumed domestically (Mazid and Kamal, 2005).

Usually, consumers prefer dry fish for their distinctive aroma and taste. Both marine and freshwater fish are used for drying in Bangladesh in different places including the coastal areas. The producers mostly buy raw materials from the landing sites, local fish markets and suppliers, then transport them to the drying yards. They use various techniques to dry fish and sell them for domestic and global markets (Islam et al., 2001). Fish drying activities start in October and end in March. In some coastal areas, fish drying begins early in September and lasts till the end of May. The process largely depends on weather and market demand (Nowsad, 2005). The sun-drying of fish involves direct exposure to the sun's energy for evaporation of water inside the fish flesh with air currents aiding vapour removal spread on bamboo racks, mats, or concrete floors. To limit microorganism growth and enzymatic activities, the moisture content of dried fish should be reduced to less than 16% (Khan, 2007; Nowsad, 2007).

Several scientists worked on different types of improved solar drying systems in Bangladesh and assessed their effectiveness in fish drying, particularly in coastal regions. It includes solar tunnel dryers, solar box dryers, hanging dryers, solar room dryers equipped with

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electric systems, etc, in the coastal regions. Most of them were made with locally available materials to minimize the costs. These improved dryers were more effective than the traditional drying system (Reza, 2002; Nowsad, 2005; Islam et al., 2006).

In Bangladesh, dried fish is commonly packed in plastic and jute bags for storage and transport. Usually, dried fish are sold in open markets, which is unsuitable for extending shelf life and maintaining the quality of the products (Rabbanee et al., 2012). Most of these packaging materials are reused before disposal (Dey et al., 2016). It was reported that, in Dhaka city, around 96% of the consumers express willingness to pay 10-15% higher prices if product quality and packaging are improved (Islam et al., 2020). However, a lack of adequate packaging and storage facilities leads to degradation and contamination of the dried products with pathogens (Remya et al., 2018). Additionally, harmful insecticides are used by some processors to prevent insect infestation during storage (Flowra et al., 2014).

Vacuum and modified atmosphere packaging (MAP) can be the alternative to maximize the quality and shelf life of dried fish products (Chowdhury et al., 2020). Vacuum and MAP are the technologies most commonly used for packaging food products in developed countries. The main purpose of this technology is to increase the shelf life and convenience of food products by preventing the growth of microorganisms and slowing down the products' biochemical processes (Babic Milijasevic et al., 2019). MAP is a non-thermal processing method that commonly uses three gases namely; carbon dioxide, nitrogen, and oxygen gas inside the pack. Their concentration depends on the type and properties of the packed food commodity (Kirtil et al., 2016). Though vacuum packaging could be an option for this purpose, this packaging is not suitable for dried fish products, particularly for small fish having visible bones or pointed edges of dried products. There is a possibility of tearing off the pack during the evacuation of air from the pack during vacuum packaging and, subsequently, pack leakage.

On the other hand, packaging materials differ in their permeability to oxygen and moisture, and choosing the appropriate packaging material is particularly vital for MAP (Forsido et al., 2021). Proper packaging acts as a passive barrier against the ingress of deteriorative vectors such as moisture, microorganisms, or oxygen and egresses desirable components such as moisture or carbon dioxide. Packaging can directly interact with the food and environment to enhance safety and quality (Rooney, 1995).

MAP technology seems to enhance the shelf life of dried fish without tearing the packaging materials depending on the gases used. Nevertheless, information regarding using this packaging technique with dried fish products is scarce. Besides, no previous research has been studied on the biochemical and microbiological changes during the storage of dried fish. Therefore, the present study focused on developing proper MAP for dried spotted snakehead fish to minimize the qualitative and quantitative losses throughout the distribution and storage phases. Implementing this technology will contribute to a year-round stable supply of high-quality dried fish products in Bangladesh.

Materials and Methods

Sample Collection and Preparation of Dried Fish

A total of 12 kg of fresh spotted snakehead fishes (*Channa puntatus*) locally called *taki* fish with an average weight of 70±20g, were collected from the local fish market in Rajshahi and brought to the Quality Control Laboratory in the Department of Fisheries of the University of Rajshahi under iced condition. First, the whole fish was de-scaled, eviscerated, and washed adequately with tap water. Then, table salt at the rate of 10% of body weight was added to the fish and kept overnight at refrigeration temperature. The next day, the salted fish was washed once to remove the adhered salts, and then turmeric powder was rubbed over the fish as a preservative. Then, the prepared fish was hung inside the hanging solar dryer installed on the roof of the building. The hanging solar dryer was a small-scale, improved dryer consisting of a 2.5×2.5×3-inch metal framed structure surrounded by a meshed net with an opening. The nets protected fishes from insects and flies. The fish was hung by the hook attached to the upper side of the dryer. Drying was continued for about a week, aiming to reduce the moisture content to less than 15%. The average temperature and humidity were measured at 28.3±2.3 ℃ and 40.5±7.0%, respectively. The fish drying was carried out in January 2021.

Packaging and Storage of Dried Spotted Snakehead Fish

Around 100 g of dried spotted snakehead fish were packed in low oxygen and moisture-permeable plastic pouches, which were multilayered (PE/ PA/PE) and transparent. MAP with various gas ratios was applied using a packaging unit (C100, Multivac, Germany) attached to a gas mixer (KM100-3 MEM, WITT, Germany). The level of O_2, N_2 and CO_2 in the headspace of the packed samples were determined by the gas analyzer (Oxy-baby M+, WITT, Germany). Four types of packaging were applied as treatments as follows: air pack (sealed without changing atmospheric gas) as control, MAP-1 (100% N_2) as treatment-1, MAP-2 (50% CO_2, 50% N_2) as treatment-2 and MAP-3 (40% CO_2, 30%O_2, 30% N_2) as treatment-3. These treatments were chosen based on previous studies and literature reviews. All the packed samples were stored in cardboard boxes at room temperature. Then, three packed samples from each packaging condition were investigated for biochemical and microbiological parameters every 15 days for 135 days.

Biochemical Analyses

The moisture level was determined by air drying a given sample in an oven at 105℃ for 24 h according to the AOAC method (AOAC, 1980). pH value of the dried fish was measured by an electrode pH-meter (HI2002-edge, Hanna inst. USA). For this purpose, dried fish was chopped into small pieces and ground using a blender. Then, 2 g of dried fish powder was homogenized with 20 mL of distilled water, and pH was measured. The TVB-N values were determined by the EC method (EC, 2005). TBARS was determined by the method with some modifications (Martínez-Zamora et al., 2020). Briefly, 2 g of ground dried fish sample was mixed with 1.5ml of 1% thiobarbituric acid (TBA) solution and 8.5 ml of 2.5% trichloroacetic acid-hydrochloric acid (TCA-HCl) using a homogenizer (IKA T-25 Digital, Germany) and kept in a water bath for 30 min at 100°C. After cooling, 5 ml of extract (with no fish) was mixed with 5 ml chloroform and centrifuged at 3500 rpm for 10 min. The absorbance of the supernatant (coloured) was measured at 532 nm using a UV spectrophotometer (1601PC, Shimadzu, Japan). The TBARS value was calculated as mg of malonaldehyde (MDA) /per kg using the formula.

TBARS (mg MDA/kg) = (Abs532/W) × 9.48.

Microbiological Analyses

Total viable counts (TVCs) were analyzed following standard plate count using plate count agar followed by the serial dilution techniques described by the (APHA, 1992). Total viable counts were converted into the logarithms of the number of colony-forming units (log $CFUg^{-1}$).

Data Analyses

All the experiments were conducted in triplicates, and the data were displayed as mean±standard deviation. The differences among treatments were analyzed using One-Way ANOVA with the Tukey test in SPSS version 20. The average values were judged significantly different as p< 0.05 was used.

Results

Changes in moisture content during storage

In the present study, the initial moisture level in dried spotted snakehead fish was 18.41% and then gradually decreased up to the 30th day of storage and remained steady with some fluctuations in the rest of the storage period under all packaging conditions. There were no such significant ($p>0.05$) variations in moisture contents among treatments in most of the sampling days. However, the control sample had significantly ($p<0.05$) higher moisture content than other samples on the 60th and 75th days of storage (Figure 1).

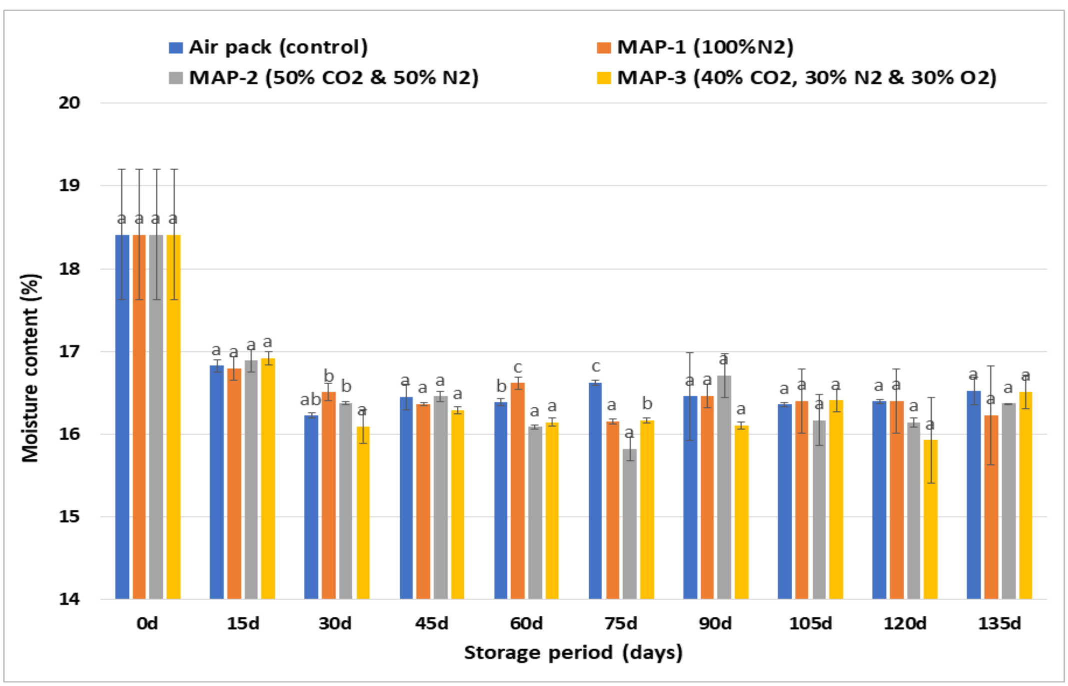

Figure 1. Changes in moisture content of dried spotted snakehead fish under different packaging conditions at ambient temperature. A statistically significant difference ($p < 0.05$) between the treatment means is shown by the distinct letters above the bars for each day.

Changes in pH Value during storage

The initial pH value of the dried spotted snakehead fish was 6.22 and increased up to the 15th day for MAP-2 & MAP-3 samples and up to the 30th day for air-pack control and MAP-1 samples and kept steady until the 75th day with some fluctuations. Then, a gradually decreasing trend was found in all samples for the rest of the storage period. During the storage time under various packaging conditions, no significant ($p<0.05$) changes were noticed in the pH values among the treatments (Figure 2).

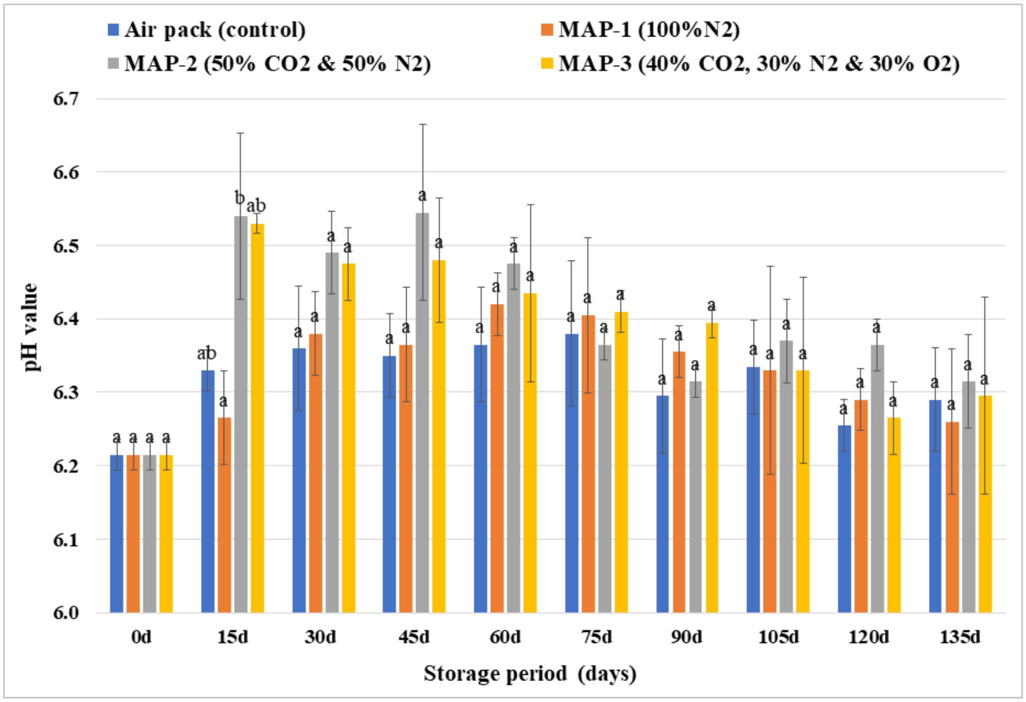

Figure 2. Changes in pH of dried spotted snakehead fish under different packaging conditions at ambient temperature. A statistically significant difference ($p < 0.05$) between the treatment means is shown by the distinct letters above the bars for each day.

Changes in total volatile base nitrogen (TVB-N) value

The initial TVB-N value of the dried spotted snakehead fish was 2.8 mg/100g and then steadily declined until the 30th day of storage before gradually increasing in the remainder of the storage period under all packaging conditions. Up to the 60th day, there were no significant ($p>0.05$) variations in TVB-N values among treatments; however, the MAP-1 sample had significantly ($p<0.05$) lower levels than the other samples for the remainder of the storage period (Figure 3). The present study observed the highest TVB-N value on the 135th day for the MAP-2 (50%CO_2 & 50%N_2) sample.

Changes in thiobarbituric acid reactive substance (TBARS) value

In the current study, the initial TBARS value of the dried spotted snakehead fish was 0.29 mg MDA/kg and steadily increased with time in all packaging conditions until the 90th day, then decreased slightly and remained steady. TBARS levels did not change significantly ($p>0.05$) among the treatments until the 105th day, but MAP-1 and MAP-2 samples had considerably ($p<0.05$) lower values than other samples for the remainder of the storage period (Figure 4).

Changes in total viable counts (TVCs)

In this study, the TVC of the dried spotted snakehead fish initially was 5.50 log CFUg^{-1} and gradually decreased until the 60th day, then slowly increased in all packaging samples for the rest of the period. However, up to the 75th day, there were no significant ($p>0.05$) variations in total viable counts (TVC) among treatments, but MAP-2 & MAP-3 had significantly ($p<0.05$) lower TVC than the others for the remainder of the period. (Figure 5). The changes in TVB-N values were aligned with changes in TVC in all packaging conditions during the storage period. Both initially decreased and showed an increasing trend (Figure 3 & 5).

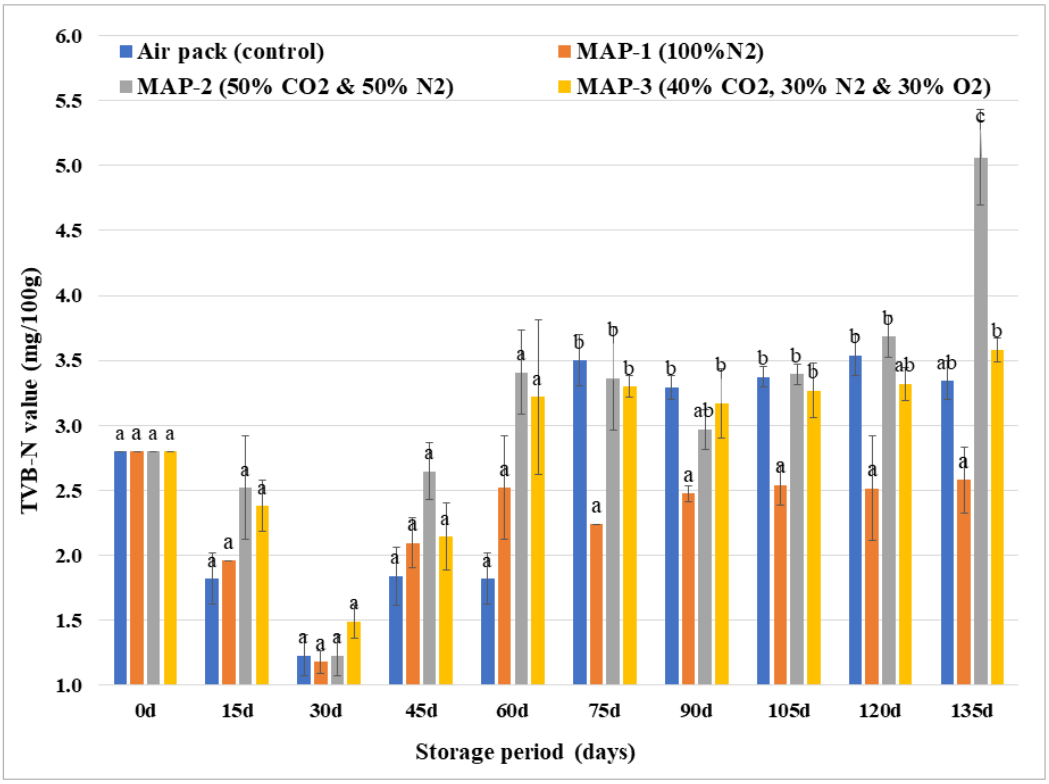

Figure 3. Changes in TVB-N of the dried spotted snakehead fish under different packaging conditions at ambient temperature. A statistically significant difference ($p < 0.05$) between the treatment means is shown by the distinct letters above the bars for each day.

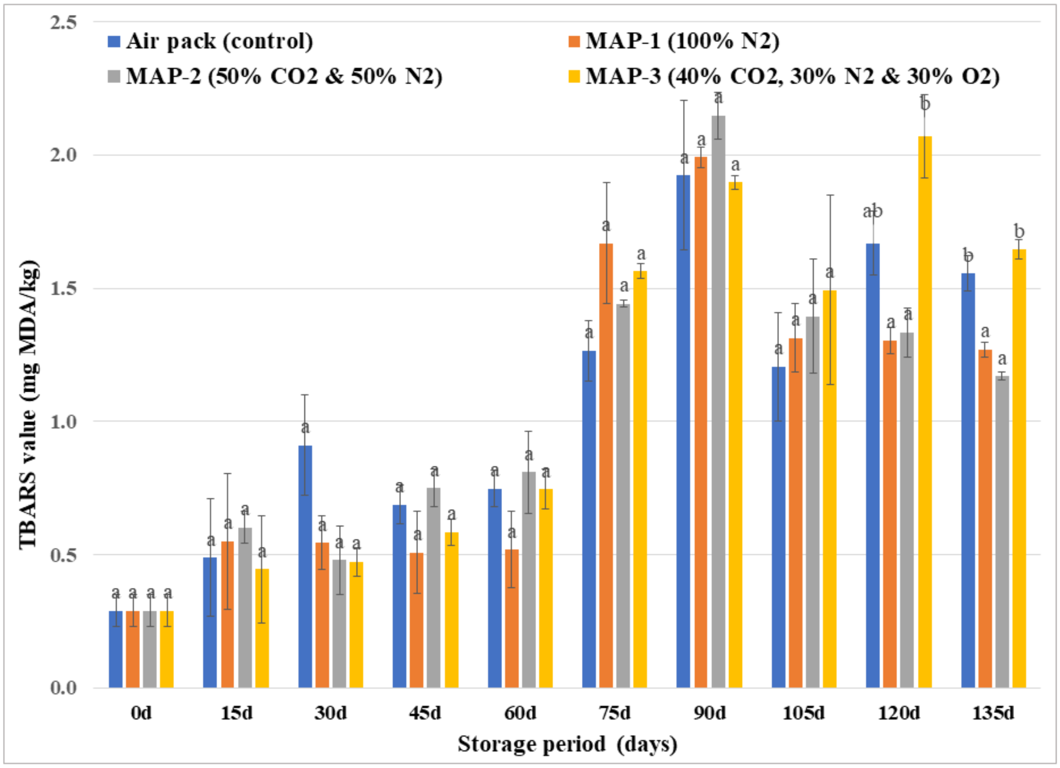

Figure 4. Changes in TBARS of the dried spotted snakehead fish under different packaging conditions at ambient temperature. A statistically significant difference ($p < 0.05$) between the treatment means is shown by the distinct letters above the bars for each day.

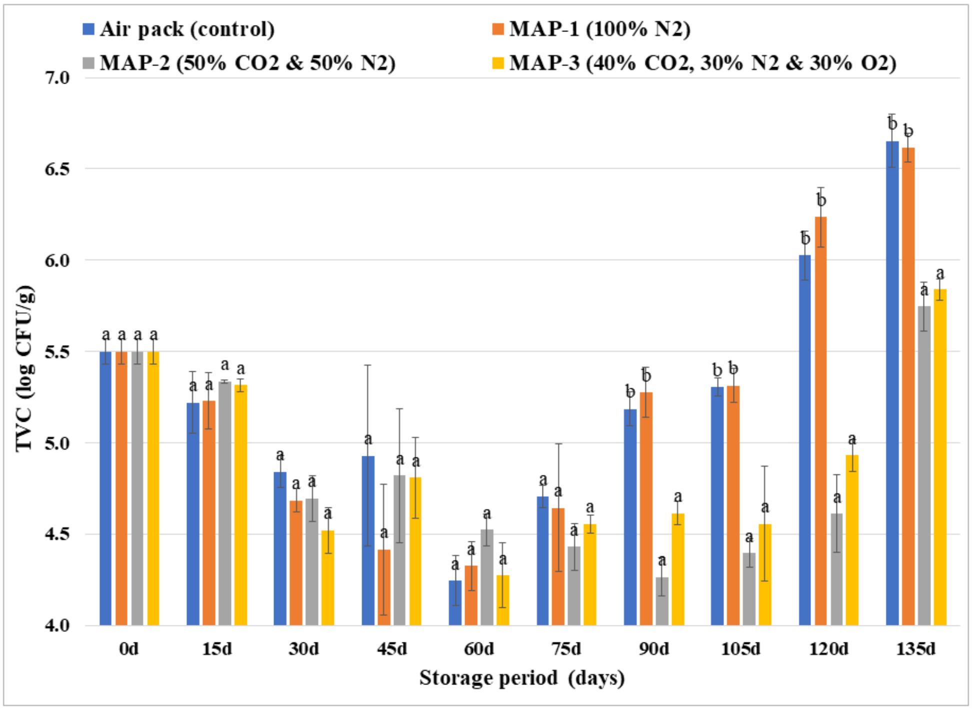

Figure 5. Changes in TVC of the dried spotted snakehead fish under different packaging conditions at ambient temperature. A statistically significant difference ($p < 0.05$) between the treatment means is shown by the distinct letters above the bars for each day.

Discussion

The present study was carried out to evaluate the quality of dried spotted snakehead products to develop MAP packaging at room temperature storage. Different quality parameters, such as moisture content, pH, TBARS, TVB-N values, and TVCs, were evaluated.

Microbial activity in food is influenced by its composition (intrinsic elements), such as water content and nutrients, as well as physical characteristics, such as temperature and environment (Gram et al., 2002). The water activity (a_w) is directly connected to water content and determines the effectiveness of inhibiting the growing microorganisms in dried fish. The final a_w values below 0.95 indicate the quality of dried fish products. According to (Frazier and Westhoff, 1978), most microbes could not grow in dried materials at less than 15% moisture level. In the present study, the initial moisture content of dried spotted snakehead products was slightly higher (18.51%) and then fluctuated between 16.92 and 15.82% during the storage period. The dried spotted snakehead fish in the market usually contain a higher moisture content (35.50%) (Rana et al., 2020). In this experiment, table salt was used at the rate of 10% of fish weight to speed up the drying process. In the final dried spotted snakehead product, 2.21±0.18 % salt was observed, which will have a significant preservative effect on dried fish during storage (Data not shown). In this case, less water must be removed to get the same result when salt is applied to the fish before drying. Generally, a water activity of 0.92 or less, comparable to a 13% NaCl content, inhibits food-borne pathogenic microorganisms (Eyo, 1993). The idea was to explain salt's preservation properties by pointing out that it has a toxic effect and prevents bacteria from obtaining moisture. Thus, it stops the growth of bacteria by plasmolyzing the cells and destroying the protoplasm of the bacteria (Fabian, 1951). Considering the salt content, the moisture content in this investigation was within permissible limits.

During storage at room temperature, the moisture content is usually increased due to the reabsorption of atmospheric moisture (Majumdar et al., 2018). In this case, water reabsorption was minimal because of using moisture barrier packaging materials, i.e., multilayer plastic pouch (PE/PA/PE). Compared to polyethylene, polyamide is a very high barrier to moisture and gas (Kjeldsen, 1993).

pH is a measurement used to determine the quality of fish and fish products. It reflects the bacterial decomposition in foods. In the present study, the pH value fluctuated between 6.22 and 6.55, which is very low and acidic. Salted dried fish have a lower pH than unsalted dried fish, regardless of the salting techniques evident in drying *Pangasius hypophthalmus* (Kumar et al., 2017). The denaturation of protein potentially causes this lower pH by adding salt to the fish before drying. As a result, it enhances the production of acidic compounds (Farid et al., 2014). As the optimal pH level for postmortem fish is usually below 6.8-7.0 (Metin et al., 2001), the pH values of all packaging conditions did not exceed the permitted limit.

The TVB-N is referred to as the total amount of ammonia (NH_3), dimethylamine (DMA), and trimethylamine (TMA) contained in the fish flesh. It is typically used to evaluate the quality of fish and fish products and a freshness index (Wu and Bechtel, 2008). Spoilage bacteria create trimethylamine. Autolytic enzymes form dimethylamine during the frozen storage, and the deamination of amino acids and nucleotide catabolites synthesizes ammonia. According to the EU directive on fish hygiene, inspectors must utilize TVB-N as a chemical check if the organoleptic inspection reveals any uncertainty about the freshness of the fish (EC, 1991).

The upper limit for TVB-N is set at 30 - 35 mg/100 g for fish and fishery products, and anything above that level is considered inappropriate for human consumption (Connel, 1995). In this study, the TVB-N values were within the permitted limit during the storage period in all samples (Figure 3). Low levels of TVB-N in the MAP-1 sample were caused by either reduced bacterial growth, decreased bacteria capacity for the oxidative deamination of non-protein nitrogen compounds, or both (Banks et al., 1980). Another investigation found a similar result where TVB-N values of dried barb fish (*Puntius* spp.) during the storage were significantly lower than the permissible limit during the storage period in all packaging samples (Chsowdhury et al., 2020). The present study observed the highest TVB-N value on the 135th day for the MAP-2 (50%CO_2 & 50%N_2) sample. The increase in TVB-N value during the storage period is mainly related to the rise in bacterial growth and other enzymes that increase the TVB-N value (Ruiz-Capillas and Moral, 2005; Islami et al., 2015).

TBARS measures the amount of malonaldehyde which is the secondary product of the oxidation of polyunsaturated fatty acids (Bremner, 2003). The second step of auto-oxidation, during which peroxide is modified, creates molecules like ketones and aldehydes (Feliciano et al., 2010). The acceptable level of TBARS is 5mg of MDA/kg of fish muscle. Beyond this level is regarded as the upper limit (Connell, 1990). In this study, the TBARS values were within the permissible level (5 mg MDA/kg) in all the packaging conditions. However, a higher TBARS value was observed on the 120th day for the MAP-3 sample. This could be due to a faster rate of secondary lipid oxidation in MAP-3 because of the increased O_2 concentration (40 CO_2 +30%O_2 + 30% N_2) sample (Arashisar et al., 2004). Another researcher also found similar results that were packed with 30% O_2 compared with no oxygen, stating that not only the O_2 but also some other bacterial enzymes participate in the oxidation process (Hernández et al., 2009). Some chemicals may be responsible for fish textural alterations, mainly when they create a covalent bond with muscle proteins (Huss, 1995). TBA is a better measure of the degree of oxidation level in fish muscles. Moreover, the TBA value does not always need to accurately reflect the current level of lipid oxidation (Özyurt et al., 2009).

The TVC value is a measurement used to determine the number of microorganisms in foods (Biyani et al., 2018). For example, during the drying of fish, the amounts of water in the fish flesh are reduced and this leads to preventing microbial growth which causes the deterioration of fish and fish products (Abraham et al., 1993). In the present study, the moisture content, TVB-N values and TVC values initially decreased up to a certain period, then either increased or steady in the rest of the storage period (Figure 1, 3 & 5). This might happen due to the adaptation of bacterial growth in a packaged environment. The upper permissible limit of total bacterial load for dried fishes is 5 logCFUg^{-1} (ICMSF, 1986). Thus, TVC values crossed the acceptable level of 5 log CFU/g^{-1} after approximately the 75th day for the air pack and MAP-1 sample, whereas around the 120th day for the MAP-2 & MAP-3 sample during the storage period. Lower bacterial load was observed in both MAP-2 and MAP-3 samples, possibly due to the bacteriostatic effects of CO_2 in MAP packaging. Several studies also showed that the effects of CO_2 concentrations delayed microbial growth, as indicated by various researchers (Stamatis and Arkoudelos, 2007; Nayma et al., 2020).

Therefore, the shelf-life of the dried spotted snakehead products was determined by considering the bacterial counts at approximately 75 days for the air pack & MAP-1 (100%N_2) sample and 120 days for the MAP-2 (50%CO_2 & 50%N_2) and MAP-3 (40%CO_2, 30%N_2 & 30%O_2) samples.

Conclusion and Recommendation

Dried fish is a natural product produced by the low-cost solar drying process considered to preserve fish worldwide. In this study, several parameters, such as moisture, pH, TBARS, and TVB-N were within acceptable limits. However, the TVC reached the allowable limit of

5 log CFUg^{-1} after the 75th day for the air pack & MAP-1 sample, whereas on the 120th day for MAP-2 & MAP-3 samples. Accordingly, MAP-2 (50%CO_2 & 50%N_2) and MAP-3 (40%CO_2, 30%N_2 & 30%O_2) exhibit the potential to enhance the quality and prolong the shelf-life of dried spotted snakehead fish products. The findings highlighted an opportunity to utilize the MAP technology in Bangladesh, offering a product with improved quality and increased shelf life. Fish processors, marketers, and other stakeholders can adopt this packaging technology to provide quality fishery products suitable for storage at room temperature.

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