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Original Article

Quality and shelf life of vacuum- and nitrogen-packed dried barb fish (*Puntius* spp.)

Paromita Chowdhury • Masudur Rahman • Syeda Nusrat Jahan • Fawzia Adib Flowra • Md. Tariqul Islam

Department of Fisheries, University of Rajshahi, Rajshahi 6205, Bangladesh

Correspondence
Md. Tariqul Islam; Department of Fisheries, University of Rajshahi, Rajshahi 6205, Bangladesh

tariqrubd@gmail.com

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Abstract

Vacuum and modified atmosphere packaging are widely used for preserving food items including fish. In this context, the quality and shelf life of air- (control), vacuum- and nitrogen-packed dried barb fish (*Puntius* spp.) stored at ambient temperature were evaluated through biochemical and microbial analyses for 90 days. In most of the storage days, significantly lower values of moisture, total volatile base-nitrogen (TVB-N) and peroxide value (PV) were observed in the vacuum pack sample compared to control. However, the TVB-N, PV, total coliforms and faecal coliforms counts were within the acceptable limit in all groups. The total plate count (TPC) increased gradually with time from an initial value of 4.29 log CFUg^{-1}. However, compared to control, significantly lower TPC were found on and after the 30th day of storage in vacuum and nitrogen pack samples. Considering the TPC value of 5 log CFUg^{-1}, the shelf life was determined at approximately 15 days for control, 35 days for nitrogen pack and 45 days for vacuum pack samples. Therefore, the vacuum pack is considered the best packaging, which may be utilised by the processors to produce and retail the dried products with prolonged shelf life.

Keywords: Dried fish; modified atmosphere packaging; *Puntius* fish; shelf life; vacuum pack.

1 | INTRODUCTION

Dried fish having a concentrated source of protein and micronutrients is well-liked and commonly eaten by people of all income groups in Bangladesh (Belton *et al.* 2014). It is one of the important sources of nutrients for poor and vulnerable rural and urban consumers of Bangladesh during the offseason of fresh fishes. It is also regarded as a cheap source of animal protein (Nowsad 2007). In Bangladesh, both marine and freshwater fishes are used to produce dried products. The coastal area plays a vital role in the drying of marine fish in Bangladesh. About 20 – 22% of marine fish are dried commercially in the coastal regions of Bangladesh (Ahmed et al. 2007). In contrast, drying of freshwater fish is generally performed in rural areas where these fishes are adequately available such as haors and beels (deep portions of seasonal floodplain area) areas mostly through traditional sun-drying by the households and small scale processors (Nowsad 2007). Among the freshwater fishes, many small indigenous fish species (SIS), including *Puntius* spp., are commonly used for drying due to easy availability (Galib *et al.* 2009; Samad *et al.* 2009).

Consumers are now very much conscious about the quality of dried fish; hence, there is a frequent complaint about the quality of dried fishes available in the market. The traditional sun-dried fish contaminated with insects and insecticides account for about 60% of the total dried products that are deemed unfit for human consumption (Nowsad 2005). Besides, considerable amounts of dried products undergo quality deterioration and contamination by pathogens due to lack of proper packaging and storage facilities (Remya *et al.* 2018). Additionally, the use of various types of health-hazardous insecticides to stop the insect infestation during storage has also reported (Flowra *et al.* 2013). Proper packaging could be an alternative to reduce the quality deterioration of these dried products. Like other products, the packaging is not developed yet for dried fishes in many developing countries (FAO 2003; Abolagba and Nuntah 2011). In Bangladesh, dried fish is usually packed in different plastic and jute bags for storage and transportation. It is generally sold in an open market without any packaging (Rabbanee *et al.* 2012). However, in the open markets of Bangladesh, dried fishes sometimes are also being sold in ordinary polythene (PE) pouch which is considered inappropriate for maintaining the quality and shelf life of the product. Since ordinary polythene is moisture permeable, the products absorb moisture and become soggy (Remya *et al.* 2018). In this case, multilayer plastics such as a combination of polythene (gas barrier) and polyamide (moisture barrier), can protect products from moisture and oxygen and thus may increase the shelf life (Dixon 2011).

Vacuum and modified atmosphere packaging (MAP) are widely used packaging systems for displaying chilled fish, meat and other products in many developed countries. It has been used effectively to increase the shelf life (by 25–400%) of the raw fillet of many fish species (Reddy *et al.* 1991). This technique, particularly the vacuum and nitrogen packaging, is applied for dried fishes in several countries including Japan, China, Singapore, Hong Kong, and some other Asian countries. Oxygen absorber packaging is also used in these countries to store and display dried products (Hooi *et al.* 1991). This type of packaging system has not yet developed for dried fishes in the markets of Bangladesh. Therefore, this study aims to develop a proper vacuum and MAP packaging to reduce the qualitative and quantitative losses of dried fishes during the distribution and retailing stage. This will eventually increase the accessibility and availability of safe and nutritious dried fishes year-round in the market.

2 | METHODOLOGY

2.1 Sample collection and preparation

Dried barb fishes (*Puntius* spp.) were collected directly from a contract processor in the Chalan Beel area of Tarash Upazila (sub-district) under the Sirajganj district, Bangladesh. In general, the availability of *P. sophore* is abundant, while *P. sarana* is common and *P. ticto* is low in Chalan Beel (Galib *et al.* 2009). Mixed species of *Puntius* were used for drying. Dried fish samples were eviscerated and washed thoroughly by the processor. The processor also treated the fish with salt at a ratio of 1:10 (salt : fish) and kept for at least 2 – 3 h to enhance the drying process and create preservative effects before drying in the solar dryer. The fishes were dried on an elevated mat made of bamboo splits and the dryer was surrounded by nylon nets to protect from flies and insects. Drying was continued for up to 3 – 4 days in November 2017. After collection, dried fishes were packed tightly in separate polythene bags and brought to the Fisheries Laboratory of the Department of Fisheries, University of Rajshahi, Bangladesh for packaging and subsequent analysis. The study was conducted from mid-November 2017 to mid-February 2018.

2.2 Packaging of dried fish

At the laboratory, 80 g of dried fish was packed in a plastic pouch, having low moisture and oxygen permeability. In this case, multi-layered, white coloured (non-transparent) pouches (polythene / polyamide / polythene) were used having a density of 130 µm. Three packaging systems were used (after Noseda *et al.* 2012), (1) air pack (control), (2) vacuum pack, and (3) Nitrogen pack with 100% N_2 gas. Air packing was performed by a typical sealer machine (Yescom, China). Vacuum and nitrogen packaging were accomplished by using a packaging machine (C 100 Multivac, Haggenmuller, Germany) attached with a nitrogen gas cylinder following the device manual. All samples were stored at ambient temperature (16 – 24°C) for three months, from mid-November to mid-February. Three packs of dried fishes (as replication) from each packaging system were subjected to biochemical and microbial analyses at every 15 days interval during the study period.

2.3 Biochemical and microbial analysis

The biochemical and microbial indices were analysed in the laboratory to reveal the quality as well as to determine the shelf life of dried barb fish. The moisture of dried fish samples (5 g) was estimated following the AOAC (1980) method by drying the fish sample in an oven at 105°C for 24 h. Total volatile base nitrogen (TVB-N) of the sample was estimated using perchloric acid (AOAC 1980). The peroxide value (PV) was estimated by the method described by Egan *et al.* (1981), which was adopted from Wood and Aurand (1977) using extracted oil from dried fish. Total plate count (TPC) expressed as colony forming units (log CFU g^{-1}) was done by a standard pour plate method of APHA (1992) by using plate count agar (Sigma-Aldrich, USA). Total fungal count (TFC) also expressed as colony forming units (log CFU g^{-1}) was determined by a standard spread plate method of APHA (1992) by using potato dextrose agar (Sigma-Aldrich, USA). In both cases, plates were incubated for 48 h at 35°C for TPC and 30°C for TFC before counting the colony. The total coliforms and total faecal coliforms were enumerated by the method described by FDA (1998) using nine tubes, the most probable number (MPN) test. In this case, only one sample from each packaging system was considered.

2.4 Data analysis

The differences among treatments were determined by one-way ANOVA along with Tukey's post-hoc test in SPSS 20 (IBM, Chicago, IL) employing an α level of significance of 0.05.

3 | RESULTS AND DISCUSSION

The present study focused on the monitoring of moisture, TVB-N value, PV and the growth of bacteria and fungus in dried barb fish stored at ambient temperature for different packaging systems.

3.1 Moisture content

Moisture content is an important quality indicator of dried fishes. In this study, the initial moisture content of dried barb fish was 14.43%. The moisture content gradually increased from the initial value up to the 45th day of storage and then decreased until the end of storage in all packaging systems (Table 1). The dried fish could absorb the moisture slowly from outside of the pack, as the plastic pouch was not impermeable but less permeable to moisture. As a result, initially, moisture content showed in increasing trend in all packaging conditions, but later the absorbed moisture was rapidly used up by the increased bacterial growth and oxidation of the products and showed a decreasing trend in moisture content. The highest moisture content of 16.36% was found on the 45th day of storage in the control air pack sample. However, compared to control, significantly lower (P < 0.05) moisture content observed on the 30th, 45th and 60th day of storage in the vacuum and nitrogen pack samples (Table 1). In the current study, the moisture content varied between 14.43 and 16.36% in all packaging types which is lower than the range of 15 to 19% observed for the same species stored in air polythene packs at different densities (Nurullah *et al.* 2007).

Microbial action in food governs by its chemical composition, for instance, nutrients and moisture content, and the physical characteristics, for example, surrounding atmosphere and temperature (Gram *et al.* 2002). In case of drying, water elimination usually inhibits the growth of spoilage microorganisms and decreases many of the deteriorative reactions governed by water (Kilic 2009). Water activity is a term directly related to the water content, which affects the efficacy in preventing microbial growth in dried fish. The final water activity (a_w) values of the quality dried fish products should be below 0.95. Generally, most of the microorganisms could not grow in dried products having moisture content below 15% (Frazier and Westhoff 1978). However, if salt is mixed with the fish before sun drying, less water requires to be eliminated to attain a similar effect. In that case, the product with a moisture content of 35 – 45%, depending on the amount of salt, will often prevent or reduce the bacterial action (Clucas 1982). In the current study, the moisture content was within the limit of acceptability in all packaging types during the storage period by considering the salt content, as 10% salt was used prior to drying.

TABLE 1 Moisture content (%) of dried barb fish in different packaging systems stored at ambient temperature (*n* = 3).

Treatments	Storage period (days)						
	0	15	30	45	60	75	90
Air pack (Control)	14.43 ±0.19^a	15.18 ±0.06^b	15.73 ±0.06^c	16.36 ±0.01^c	15.56 ±0.11^b	15.15 ±0.12^b	15.46 ±0.10^a
Vacuum pack	14.43 ±0.19^a	15.29 ±0.06^b	15.39 ±0.03^b	15.46 ±0.04^b	15.07 ±0.04^a	14.57 ±0.35^a	15.25 ±0.81^a
Nitrogen pack	14.43 ±0.19^a	14.63 ±0.06^a	14.86 ±0.12^a	15.30 ±0.04^a	15.21 ±0.12^a	15.16 ±0.03^b	15.10 ±0.03^a

Values are Mean ± SD. Values in the same column with different superscript letters represent the significant difference (P < 0.05).

3.2 Total volatile base nitrogen (TVB-N)

The TVB-N is an important indicator for the quality assessment of fish and fishery products. TVB-N is the totality of the amount of trimethylamine (TMA), ammonia (NH_3) and dimethylamine (DMA) in fish, which is frequently used as a spoilage indicator of fish (Wu and Bechtel 2008). In general, the TMA is originated by spoilage bacteria, NH_3 is formed by the deamination of amino acids and nucleotide catabolites, and DMA is formed by autolytic enzymes (Huss 1995). As per the European Union directive on fish hygiene, if there is any doubt on the organoleptic examination for the freshness of fish, TVB-N must be used as a chemical check by the inspector (EU 1991).

In this study, the initial TVB-N value was 2.8 mg $100g^{-1}$ in dried barb fish. A gradual increase was observed in air and vacuum pack samples in the storage period. For nitrogen pack, the TVB-N value showed an increasing trend up to the 30th day and then remained almost steady until the end of the storage period (Table 2). Compared to control, TVB-N values were significantly lower (P < 0.05) in the vacuum pack and nitrogen pack samples during most of the storage period. Moreover, significantly (P < 0.05) higher TVB-N values were also found in the nitrogen pack sample on 30, 45, 60 and 75th day of storage in comparison to the vacuum pack sample. The nitrogen pack may contain 1 – 2% oxygen, as 100% nitrogen flushing could not be achieved during packaging. This lower amount of oxygen may allow the growth of some aerobic bacteria and this resulted in an increased TVB-N value in the nitrogen pack sample compared to the vacuum pack sample. Overall, lower TVB-N values were seen in the vacuum pack sample compared to others (Table 2).

TABLE 2 Total volatile basic nitrogen values (mg $100g^{-1}$) of dried barb fish in different packaging groups stored at ambient temperature (n = 3).

Treatment	Storage period (days)						
	0	15	30	45	60	75	90
Air pack (Control)	2.80± 0.28^a	8.17± 0.40^b	8.27± 0.11^b	10.43± 0.44^b	11.38 ±0.37^c	11.84 ±0.08^c	12.08 ±0.06^c
Vacuum pack	2.80± 0.28^a	3.50± 0.08^a	4.18± 0.08^a	4.54± 0.48^a	6.15± 0.24^a	7.07± 0.30^a	9.58± 0.31^b
Nitrogen pack	2.80± 0.28^a	3.72± 0.25^a	8.56± 0.15^b	9.14± 0.37^b	9.50± 0.08^b	8.66± 0.01^b	7.56± 0.40^a

Values are Mean ± SD. Values in the same column with different superscript letters represent the significant difference (P < 0.05).

According to Kimura and Kiamakura (1934), the TVB-N value of 35 – 40 mg $100g^{-1}$ is regarded as the upper acceptable limit for dried fish. Above that level, the fish products are considered unfit for human consumption. Considering that limit, the TVB-N values of dried barb fish were far below the acceptable limit during the storage period in all packaging types. Low levels of TVB-N value in dried fish samples might be due to either a decreased bacterial number or reduced capacity of those bacteria for the oxidative deamination process of non-protein nitrogenous compounds or both (Banks *et al.* 1980).

3.3 Peroxide value (PV)

Peroxide value is commonly used as an important indicator to assess the degree of primary oxidation of lipid (Masoud *et al.* 2008) and values are expressed as millimoles or milliequivalents of active oxygen per kg of fat. Peroxide value measures the quantity of hydroperoxides formed in the fish body. The acceptable limit of peroxide value is 10 – 20 mEq kg^{-1} of fish oil (Connell 1995).

The initial PV of dried barb fish was 2.08 mEq kg^{-1} fish oil. In case of air and nitrogen pack samples, the PV gradually increased during the entire storage period except for a decrease on the 90th day for nitrogen pack sample. On the other hand, the PV gradually increased up to the 30^{th} day of storage in the vacuum pack sample and then slowly decreased until the end of the storage period (Table 3). In this study, the initial rise of PV was due to the development of hydroperoxides which has been increased at the initial stage of oxidation but decreased at the later stage when the degree of cleavage and reactions surpass the hydroperoxide formation (Underland *et al.* 1999; Cyprian *et al.* 2015). There was no significant (P > 0.05) difference observed among the treatments until the 30^{th} day of storage. For the remaining storage period, significantly lower (P < 0.05) PVs were observed in the vacuum pack samples than the control and nitrogen pack samples (Table 3). Comparatively lower PV was observed in the vacuum pack sample than others.

TABLE 3 Peroxide values (mEq kg^{-1} fish oil) of dried barb fish in different packaging groups stored at ambient temperature (n = 3).

Treatments	Storage period (days)						
	0	15	30	45	60	75	90
Air pack (Control)	2.08± 0.06^a	3.54± 0.20^a	3.92± 0.06^a	4.35± 0.07^b	5.44± 0.20^c	5.22± 0.06^b	6.76± 0.08^b
Vacuum pack	2.08± 0.06^a	3.13± 0.04^a	3.87± 0.13^a	3.23± 0.04^a	3.36± 0.08^a	3.69± 0.13^a	3.05± 0.07^a
Nitrogen pack	2.08± 0.06^a	3.44± 0.06^a	4.10± 0.14^a	4.78± 0.11^c	4.59± 0.13^b	5.51± 0.16^b	2.90± 0.16^a

Values are Mean ± SD. Values in the same column with different superscript letters represent the significant difference (P < 0.05).

Vacuum packaging can interrupt the lipid oxidation of foods by limiting the availability of oxygen, which creates a preservative effect on keeping the quality of muscle foods (Etemadian *et al.* 2012). This packaging has been observed to decrease the oxidative spoilage significantly in frozen fish and fishery products (Taheri and Motallaabi 2012). This is in agreement with the present study, where lower PV is observed in the vacuum pack sample than others. In case of dried fish products, quality deterioration occurs by microbial growth and lipid oxidation during the storage period (Doe 2002).

In this study, the PVs in all samples were far below the acceptable limit during the entire storage period. The PV provides a measure of the initial phases of oxidative rancidity, which does not associate with rancidity (Connell 1995; Huss 1995). However, the value over the 20 mEq kg^{-1} fat, the fish and fishery products can produce a rancid smell and taste (Connell 1995).

3.4 Total pate count (TPC)

Total bacterial count is an important measure for the assessment of the microbial quality of a product as well as for the shelf life estimation. Microbial activity in food depends on its nutritional compositions along with the physical parameters, for instance, surrounding atmosphere and temperature (Gram *et al.* 2002). Dehydration of products prevents the growth of spoilage microorganisms (Kilic 2009). Uncontrolled growth of microorganisms in such dried products may lead to severe consequences in keeping the quality and safety of the product (Abraham *et al.* 1993).

The initial TPC of dried barb fish was 4.29 log CFU g^{-1}. In a previous study conducted by Islam *et al.* (2013), the TPC was reported 5.36 log CFU g^{-1} in traditionally dried barb fish collected from the same area. The TPC gradually increased in all packaging types throughout the study period. Comparatively lower TPCs were observed in vacuum and nitrogen pack samples than control samples throughout the storage period. However, significantly ($P < 0.05$) lower TPCs were found on and after the 30th day of storage in vacuum and nitrogen pack samples when compared to the control samples (Table 4).

TABLE 4 Total plate count (log CFU g^{-1}) of dried barb fish in different packaging groups stored at ambient temperature (n = 3).

Treatments	Storage period (days)						
	0	15	30	45	60	75	90
Air pack (Control)	4.29± 0.14 ^{a}	5.01± 0.07 ^{a}	6.06± 0.25 ^{b}	6.45± 0.14 ^{c}	6.66± 0.14 ^{b}	7.01± 0.04 ^{c}	6.88± 0.15 ^{b}
Vacuum pack	4.29± 0.14 ^{a}	4.78± 0.14 ^{a}	4.92± 0.08 ^{a}	5.00± 0.17 ^{a}	5.56± 0.15 ^{a}	5.64± 0.03 ^{a}	6.01± 0.16 ^{a}
Nitrogen pack	4.29± 0.14 ^{a}	4.67± 0.16 ^{a}	4.93± 0.08 ^{a}	5.12± 0.15 ^{a}	5.67± 0.13 ^{a}	6.16± 0.08 ^{b}	6.29± 0.07 ^{a}

Values are Mean ± SD. Values in the same column with different superscript letters represent the significant difference (P < 0.05).

In the current study, the TPC exceeded the 5 log CFU g^{-1} on the 15th, 35th and 45th day in air pack (control), nitrogen pack and vacuum pack samples respectively which is considered the upper limit of acceptability for dried fishes (ICMSF 2002; Figure 1). Several studies have indicated that the bacteria grow more rapidly in fish in air packed than that of vacuum packed (Lyon and Reddmann 2000; Özogul *et al.* 2004). Therefore, according to the total bacterial counts, the shelf life of dried barb fish was estimated at approximately 15 days for air pack, 35 days for nitrogen pack and 45 days for vacuum pack samples (Figure 1). Sophonphong (1991) observed a similar trend where air packed and nitrogen packed dried salted sardines *Sardinops neopilchardus* were unacceptable after the fourth week of storage whereas the vacuum packed samples remained acceptable for storage at room temperature for up to 12 weeks. Better performance was observed for vacuum pack sample to keep the quality for an extended period. The higher initial bacterial counts might be the reason for the limited shelf life of dried fish in this study.

3.5 Total fungal count (TFC)

There was no TFC (yeast and moulds) observed by the applied method in any samples. Fungal growth and production of fungal toxin in the fish favoured by the hot and humid climate, moisture content of >16% and insect damage (Hamblin 2000). Since the moisture content did not exceed 16% during storage, the fungal attack could not be successful in samples.

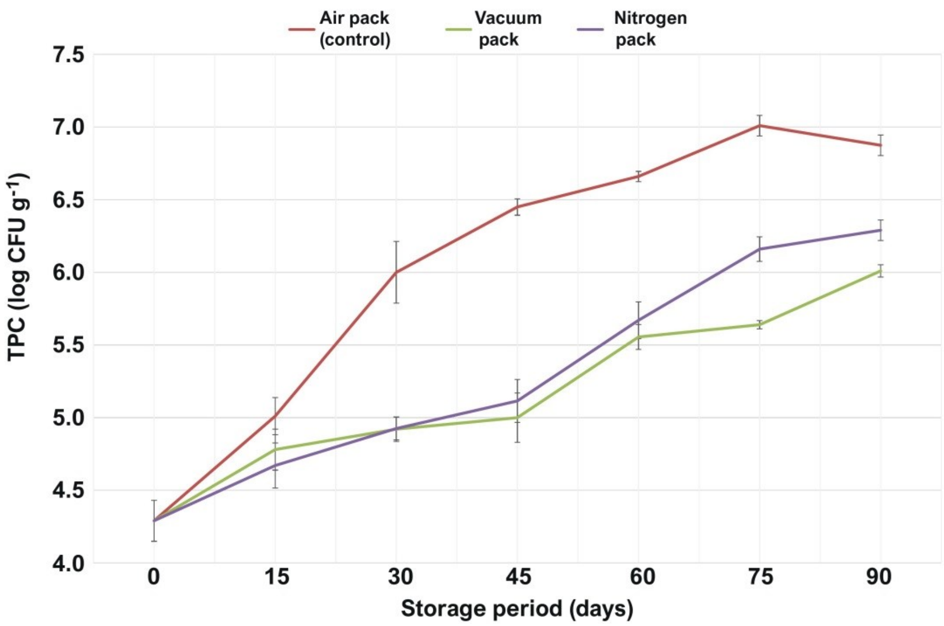

FIGURE 1 Changes in total plate count (log CFU g^{-1}) of dried barb fish in different packaging groups during storage at ambient temperature (n = 3).

3.6 Total coliforms and faecal coliforms

Total coliforms bacteria are usually observed in the aquatic environment, in soil and on vegetation and are commonly harmless. Faecal coliforms bacteria are a sub-group of total coliforms bacteria abundant in the intestines and faeces of humans and animals. They are regarded as sanitary index organisms for foods and water. Their presence in the food indicates the probable occurrence of pathogenic bacteria of faecal origin (Li and Liu 2019). The acceptable limits of total coliforms and faecal coliforms are <100 MPN g^{-1} and <10 MPN g^{-1} respectively for fish and fishery products (ICMSF 1986).

In this study, total coliforms and faecal coliforms were found only on the 45th and 75th day of storage in all packaging groups. The total coliforms count of dried barb fish ranged from 3.6 – 93 MPN g^{-1} and faecal coliforms count ranged from 3.6 – 9.2 MPN g^{-1} in all packaging groups during the study period (data not shown). The presence of those bacteria on the particular sampling day might be due to the rough handling during packaging. However, the total coliforms and faecal coliforms counts were within the acceptable limit in samples in all packaging groups which indicates that the samples were collected from the non-polluted area and produced in aseptic conditions by the dry fish processors and handlers. The lower number of coliforms can be valuable for pointing out the effectiveness of safety measures taken during processing and handling (Elhadi *et al.* 2004).

4 | CONCLUSIONS

Dried fish are becoming a favourite day by day for a large number of people in Bangladesh and other Asian countries for its delicious taste. However, the storage and display systems are inadequate to ensure quality of the dried fish products. Vacuum and modified atmosphere packaging have shown as a proper packaging technique to increase the shelf life of a wide range of fishery products. In the present study, the moisture content, TVB-N, PV and total coliform counts and faecal coliform counts of the samples in all packaging groups were within the acceptable limit throughout the storage period. However, the TPC exceeded the acceptable limit during the storage period. Considering the bacterial counts, the shelf life of dried barb fish was determined between 15 days and 45 days. The overall performance was better for vacuum pack samples than others. However, further research needs to be done on modified atmosphere packaging with a different combination of gases to find out the best combinations for extended shelf life.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

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CONTRIBUTION OF THE AUTHORS

MTI, FAF & **SNJ** research design;
PC & **MR** primary data collection through laboratory analysis;
PC & **MTI** data analysis and draft MS preparation;
SNJ & **FAF** critical review of the manuscript;
MTI research supervision.

P Chowdhury https://orcid.org/0000-0001-8148-1958
M Rahman https://orcid.org/0000-0001-6574-9117
SN Jahan https://orcid.org/0000-0003-4902-4882
FA Flowra https://orcid.org/0000-0002-3674-391X
MT Islam https://orcid.org/0000-0003-1623-5210