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Shelf-life of vacuum and modified atmosphere packaged sliced pangasius catfish (*Pangasianodon hypophthalmus*) under refrigerated storage condition

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Abstract. The effects of vacuum (VP) and 100% N_2 modified atmosphere packaging (MAP) on the quality and shelf-life of sliced pangasius catfish (*Pangasianodon hypophthalmus*) during refrigerated storage (4°C) were investigated up to 12 days. The values of pH, total volatile base nitrogen (TVB-N) and thiobarbituric acid reactive substance (TBARS) of sliced fish samples during storage under VP and MAP packaging were within the limit acceptable for chilled fish. Total viable count (TVC) of pangasius fish, on the other hand, gradually increased from the initial value of 4.32 ± 0.04 to 8.30 ± 0.13 log CFU/g on day 9 for non-sealed pack (control) and 7.64 ± 0.12 and 8.34 ± 0.07 log CFU/g for VP and MAP on day 12. There were no significant ($p < 0.05$) differences in TVC values among the three packaging conditions during the storage period except on day 9 where significantly ($p < 0.05$) lower TVC values were observed in the VP sample compared to that of other samples. Based on the bacterial counts of 7 log CFU/g, which is considered as the upper acceptable limit for fresh and frozen fish, the shelf-life was determined as the excess of 6 days for control pack and MAP samples, and excess of 9 days for VP sample. Therefore, VP is a good option to increase the shelf-life of wet fish, which can be adopted by the superstores to display their products with extended shelf-life.

Keywords: Pangasius fish, Vacuum, MAP, Shelf-life

Introduction

Among the exotic fish species introduced in Bangladesh, catfish (*Pangasianodon hypophthalmus*) has become very popular among freshwater aquaculturists due to their easy culture system, resistance to impaired water quality and adaptability to intensive aquaculture high yield and low production cost, good taste, fewer intramuscular bones and high consumer acceptance (Sarker 2000). Total pangasius production in Bangladesh reached to 0.45 million metric tons in 2017–18, making it the second-largest single species contributor after hilsa (DoF 2018). Recently, its market price became very low compared to the production cost that discourages the farmer to culture this fish in Bangladesh. In this case, the development of different value-added products from pangasius fish may act as an alternative way to increase the value of fish (Viji *et al.* 2015).

Food preference has been changing with the social and economic development of the country. Now city-dwellers especially working mothers and housewives seek ready-to-cook (RTC) or prepared foods instead of raw ingredients in their busy life. Presently there is no such fishery product in the domestic market. In Bangladesh, fishes are usually sold as a whole with no proper storage and display facilities. As a result, considerable amounts of raw fishes undergo quality deterioration during retailing (Hossain *et al.* 2013). On the other hand, many retail superstores of the country now display the fresh fishes as whole fish, sometimes as whole dressed and hardly as portions under refrigeration or icing condition. As a result, consumers are restricted to buy the affordable portions of fishes, particularly for larger popular fishes. This chilled storage will not necessarily extend the shelf-life sufficiently for retail distribution and display purposes. Repeated thawing and freezing is another problem which is being practiced in retail superstores. As a consequence, the quality of the fishes is deteriorated, and a considerable portion is spoiled, which is one of the reasons for high pricing in those superstores. Fresh fishes are highly perishable products due to their biological composition. Even under normal refrigerated storage, the shelf-life of the products is short due to enzymatic, chemical and microbial spoilage. However, with increasing consumer demands of fresh products with prolonged shelf-life and rising energy costs associated with freezing and frozen storage, the fish-processing industry and retail superstores are actively seeking an alternative method for the marketability of fresh, refrigerated fish and at the same time economizing on energy costs (Ashie *et al.* 1996).

Vacuum and modified atmosphere packaging are widely used packaging techniques used for displaying chilled fish, meat and their various products in developed countries. In vacuum packaging, the product is placed inside a type of plastic pouch having low permeability to oxygen, and the air is exhausted causing the bag to collapse around the product before it is sealed (Adams and Moss 2008). The gaseous atmosphere of vacuum packaging is changed, but it is probably altered during storage, thus considered modified due to a 10–20% increase in the CO_2 amount produced by microbial activity utilizing residual oxygen. This CO_2 may inhibit the growth of aerobic microflora generally associated with the spoilage of fish and fish products (Silliker and wolfe 1980). Modified atmosphere packaging (MAP) is a preservation method by altering the atmospheric environment around a perishable food with a single or a mixture of protective gas (Arashisar *et al.* 2004). MAP has been used successfully to extend the shelf-life (25–400%) of the raw fillets of many fish species at refrigeration temperatures (Reddy *et al.* 1991, Pantazi *et al.* 2008). It allows retailers to sell fresh fishes at refrigeration conditions for an extended period. The growth of common spoilage bacteria is inhibited, and a microaerophilic group of lactic acid bacteria become the dominant spoilage organisms (Soccol and Oetterer 2003). Nitrogen (N_2) is used for dislocating the oxygen from the packaging, decreasing oxidative rancidity and inhibiting the growth of aerobic microorganisms. For its low solubility, it is used as a filler gas preventing the possible packaging collapse caused by the accumulation of CO_2. Nitrogen also helps to prevent mould growth and insect attacks. It can indirectly influence the microorganisms in perishable foods by retarding the growth of aerobic spoilage organisms (Farber 1991, Phillips 1996). To ensure increased shelf-life and keeping quality, vacuum and MAP offer technological options to the fishery products. MAP lengthens the shelf-life of a product and reduces economic losses. This technology allows delivering the product to long-distance markets; therefore, the commercial value may increase (Pastoriza *et al.* 1996). This type of packaging system has not yet been developed in Bangladesh. The objective of this research was to determine the overall quality and shelf-life of sliced pangasius catfish under vacuum and MAP stored at 4°C.

Materials and Methods

Sample collection and preparation: Live pangasius catfish (*Pangasianodon hypophthalmus*) with an average size of 1 kg were purchased from the local market and brought to the Quality Control Laboratory of Department of Fisheries, Rajshahi University. Upon arrival, fishes were washed with tap water at room temperature. Then the fishes were dressed and cut into around 0.5 inch thick slices of having approximately 50 g of weight. Slices were washed two times with tap water at room temperature and the final wash was done with distilled water. At that time, the fish slices became ready for packaging.

Packaging and storage of samples: The required amount of sliced fishes was packed under vacuum and MAP in low gas and moisture permeable plastic pouches. The packaging material used for this purpose was a multi-layered transparent pouch (PE/PA/PE), having 100 μm thickness. Three types of packaging were applied under vacuum and MAP using the method described by Noseda *et al.* (2012). Those three types of packaging were used as treatments namely, (1) aerobic, not sealed pack as control; (2) vacuum pack as treatment-1; and (3) MAP with 100% N_2 pack as treatment-2. Vacuum and MA packaging was performed using a packaging machine (C100 Multivac, Haggenmuller, Germany) attached with a nitrogen gas cylinder according to the manufacturer's instruction. All the packaged samples were stored at 4°C in a laboratory refrigerator. Three samples from each packaging conditions were examined at storage days 0, 3, 6, 9, and 12 in the laboratory.

Biochemical and microbiological analyses: Biochemical and microbiological parameters were analysed at the laboratory to evaluate the quality of fish slices as well as to determine the shelf-life under refrigerated storage. The pH value of the fish flesh homogenate was measured by means of a glass electrode pH meter (HI2002-Edge, Hanna Inst, USA). In this case, 10 g of cut flesh was homogenized with 50 mL of distilled water to make the homogenate. Total volatile base nitrogen (TVB-N) was determined using 10 g of ground fish sample with perchloric acid according to EC (2005) method. Thiobarbituric acid reactive substances (TBARS) values were measured by colourimetric method using a spectrophotometer (1601PC, Shimadzu, Japan) according to the procedure of Witte *et al.* (1970). TBARS values was calculated as follows: TBARS value (mg malonaldehyde/kg) = optical density (O.D.) $\times$ 5.2. Total viable count (TVC) was determined by a standard plate count method on plate count agar following APHA (1992) method. Plates were incubated at 35°C in an incubator for 48 h and colonies were counted.

Statistical analysis: The values were expressed as mean $\pm$ standard deviation. Differences among treatments were estimated by using one-way ANOVA with the application of a Tukey test using SPSS version-20 (IBM, Chicago, IL). Average values were considered significantly different when $p < 0.05$.

Results and Discussion

The present study was undertaken to assess the shelf-life of sliced pangasius catfish (*P. hypophthalmus*) under vacuum and modified atmosphere packaging at 4°C. Various biochemical parameters namely; pH, TVB-N, TBARS value and bacterial load in terms of TVC were monitored during 12 days of storage period.

Changes in *pH:* Muscle pH is an indicator of the extent of microbial growth in fish; some proteolytic bacteria can produce acid after decomposition of carbohydrates, thereby increasing the acid level of the fish. The pH of freshwater fish flesh in the fresh condition is almost neutral (Virta 2009). The decomposition of nitrogenous compounds leads to an increase in the pH in the fish flesh at the post-mortem period (Shenderyuk and Bykowski 1989). The post-mortem pH acceptability limit is usually 6.8 ~ 7.0 (Metin *et al.* 2001). Changes in muscle pH of refrigerated sliced pangasius fishes under different packaging conditions are shown in Table I. The initial pH was 6.68 ± 0.13 and then decreased up to 3rd day of storage for control and vacuum packed and 6th day for N_2 packed samples. After that, the pH values gradually increased with some fluctuation sunder all packaging conditions during the storage period. However, significantly ($p < 0.05$) lower pH values were observed on the 6th and 9th day of storage in all packed samples compared to those of control (Table I). The pH values were within the acceptable limit in all packaging conditions during the storage period. Generally, the low muscle pH of muscle post-mortem is associated with the quality changes in fish (Kramer and Peters 1981, Reza et al. 2009). There is a direct relation between TVB-N and pH value and the microbial spoilage, which leads to autolysis in the fish tissue. Changes in pH, microbial growth, trimethylamine and volatile protein nitrogen have been used as indices of the freshness of iced aquatic species (Cheuk *et al.* 1979).

Table I. pH value of sliced pangasius fishes under different packaging conditions at refrigerated storage

Treatments	Storage period (days)				
	0	3	6	9	12
Not sealed pack (Control)	6.68 ± 0.13^a	6.40 ± 0.31^a	6.90 ± 0.03^c	6.71 ± 0.25^b	6.82 ± 0.15^a
Vacuum pack	6.68 ± 0.13^a	6.03 ± 0.26^a	6.48 ± 0.17^b	6.04 ± 0.14^a	6.48 ± 0.14^a
Nitrogen (100%) pack	6.68 ± 0.13^a	6.38 ± 0.05^a	5.99 ± 0.18^a	6.19 ± 0.10^a	6.55 ± 0.39^a

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

The initial declining trend of pH probably occurred due to the accumulation of lactic acid by anaerobic glycolysis and the liberation of inorganic phosphates by the degradation of ATP. This is in agreement with the results reported by Ayala *et al.* (2010) for sea bream during 22 days of ice storage. On the other hand, the increasing trend of pH at later stages of storage may be occurred due to the production of amines and other volatile bases by the autolytic and microbial action on protein and other components of fish (Wang and Brown 1983, Binsi *et al.* 2007). Similar increasing behaviour was observed during storage in brined chub mackerel at refrigerated temperature (Goulas and Kontominas 2005).

Changes in TVB-N: TVB-N is an important characteristic for the assessment of the quality of fish and fishery products. The combination of the total amount of ammonia (NH_3), di- and tri-methylamine in fish is called total volatile base (TVB) nitrogen and is commonly used as an index of fish freshness (Wu and Bechtel 2008). European Union directive on fish hygiene specifies that if the organoleptic examination shows any doubt as to the freshness of the fish, inspectors must use TVB-N as a chemical check (Castro *et al.* 2006). The acceptable limit of TVB-N value for freshly caught fish is 5.6 mg/100g, and ice-stored fish is 30–35mg/100g of fish (Connell 1995).

In the present study, the initial TVB-N value of pangasius fish slices was 2.38 ± 0.20 mg/100g, and then, the values gradually increased with progressing storage time under all packaging conditions. The range of TVB-N value was 2.38 ± 0.20 to 5.18 ± 0.99 mg/100g during the storage period. No significant ($p > 0.05$) differences were observed in relation to TVB-N values among three packaging conditions. However, the values were within the acceptable limit of 30–35 mg/100g for iced fish in all packaging conditions (Table II). A more or less similar result was reported by Soccol *et al.* (2005) where there were no significant differences on TVB-N values among the treatments of tilapia fish (air, vacuum, and a MAP with 60% CO_2/40% O_2) during the 20 days of storage at 1°C. The amount of TVB-N in fish increases as spoilage progresses. An increase in TVB-N during storage is a consequence of the liberation of basic compounds by microbial activity on protein and non-protein nitrogenous compounds. As a result, the pH also increased at later stages of storage due to the production of basic components such as ammonia, dimethylamine, trimethylamine, other biogenic amines (Goulas and Kontominas 2007).

Table II. TVB-N value (mg/100g) of sliced pangasius fishes under different packaging conditions at refrigerated storage

Treatments	Storage period (days)				
	0	3	6	9	12
Not sealed pack (Control)	2.38±0.20[a]	2.24±0.79[a]	2.66±0.20[a]	3.64±0.40[a]	5.01±0.29[a]
Vacuum pack	2.38±0.20[a]	2.21±0.61[a]	2.52±1.19[a]	3.64±1.19[a]	4.62±0.59[a]
Nitrogen (100%) pack	2.38±0.20[a]	1.68±0.40[a]	1.96±1.19[a]	5.18±0.99[a]	4.34±0.20[a]

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

Changes in TBARS value: TBARS is a measure of one of the secondary lipid oxidation products, the malonaldehyde. The results are expressed with regard to the standard malonaldehyde used and reported as micromoles malonaldehyde present in 1 g of fat. The acceptable limit of TBARS value is 2 mg malonaldehyde/kg fish. However, beyond this limit, an objectionable odour and taste develop in fish (Connell 1990). Therefore, it can be used as a quality indicator of food products. In the present study, the initial TBARS value was 0.30±0.09 mg malonaldehyde/kg of sliced pangasius fish, and then, values increased rapidly for not sealed pack, and slowly for vacuum and nitrogen packed samples during refrigerated storage. Significantly ($p<0.05$) lower TBARS values were observed on the 6^{th}, 9^{th}, and 12^{th} day of storage in the vacuum pack sample compared to that of the control sample (Table III). However, the TBARS values were within the acceptable limit (2 mg malonaldehyde/kg) in all samples during the storage period. Sallam *et al.* (2006) observed almost similar initial TBARS value (0.37 mg malonaldehyde/kg) in fresh raw Pacific saury. In the present study, lower TBARS values were observed in the case of vacuum and nitrogen pack samples due to the absence of oxygen in those packs. According to Aubourg (1993), TBARS records may not reveal the actual rate of lipid oxidation as malonaldehyde may interact with other components of fish muscle such as amines, nucleosides, and nucleic acid, proteins, amino acids of phospholipids, and other aldehydes that are end products of lipid oxidation and this interaction may vary significantly with fish species. The usefulness of thiobarbituric acid (TBA) value and Thiobarbituric acid reactive substances (TBARS) as an indicator of shelf-life depends on the type of product (DeWitt and Oliveira, 2016). Alfaro et al. (2013) determined that TBA was not a useful indicator for shelf-life determination of MAP of Atlantic horse mackerel, while Torrieri *et al.* (2011) found TBARS to be a critical index for determination of shelf-life of MAP of Bluefin tuna.

Table III. TBARS value (mg malonaldehyde/kg) of sliced pangasius fishes under different packaging conditions at refrigerated storage

Treatments	Storage period (days)				
	0	3	6	9	12
Not sealed pack (Control)	0.30±0.09[a]	0.33±0.06[a]	0.67±0.09[b]	0.93±0.04[c]	1.12±0.03[b]
Vacuum pack	0.30±0.09[a]	0.25±0.06[a]	0.37±0.13[a]	0.44±0.08[b]	0.58±0.04[a]
Nitrogen (100%) pack	0.30±0.09[a]	0.17±0.09[a]	0.40±0.10[ab]	0.23±0.07[a]	0.64±0.05[a]

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

Changes in TVC: TVC is an important measure for the assessment of the microbial quality of a food product. The initial TVC of sliced pangasius fish was 4.32±0.04 log CFU/g on plate count agar that indicated an acceptable initial quality of fish. Most of the available literature on freshly caught freshwater fishes (seabass, tilapia, rainbow trout, and silver perch) reported bacterial counts of 2–6 log CFU/g (Gelman *et al.* 2001). In the present study, the TVC gradually increased with the progression of storage time in all packaging conditions. There were no significant differences in TVC observed among all packaging conditions until the 6th day of storage. However, significantly ($p<0.05$) lower TVC was observed on the 9th, and 12th day of storage in the vacuum pack sample compared to that of other samples (Table IV).

Table IV. Total viable count (log CFU/g) of sliced pangasius fishes under different packaging conditions at refrigerated storage

Treatments	Storage period (days)				
	0	3	6	9	12
Not sealed pack (Control)	4.32±0.04[a]	4.26±0.12[a]	6.24±0.28[a]	8.30±0.13[b]	-
Vacuum pack	4.32±0.04[a]	5.44±0.53[a]	5.83±0.55[a]	6.87±0.41[a]	7.64±0.12[a]
Nitrogen (100%) pack	4.32±0.04[a]	4.93±0.38[a]	6.63±0.19[a]	7.91±0.24[b]	8.34±0.07[b]

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

The TVC values exceeded the 7 log CFU/g, which is considered as the upper acceptable limit for fresh and frozen fish and cold-smoked fish species (ICMSF, 1986) after 6th day for not sealed pack (control), after 9th day for vacuum pack, and after 6th day of storage for nitrogen pack sample (Fig. 1). Taking the 7 log CFU/g as the maximum acceptable limit for fresh, frozen and cold-smoked fish species, the shelf-life of sliced pangasius fish was determined at the excess of 6 days for not sealed pack & nitrogen pack sample and excess of 9 days for vacuum pack sample.

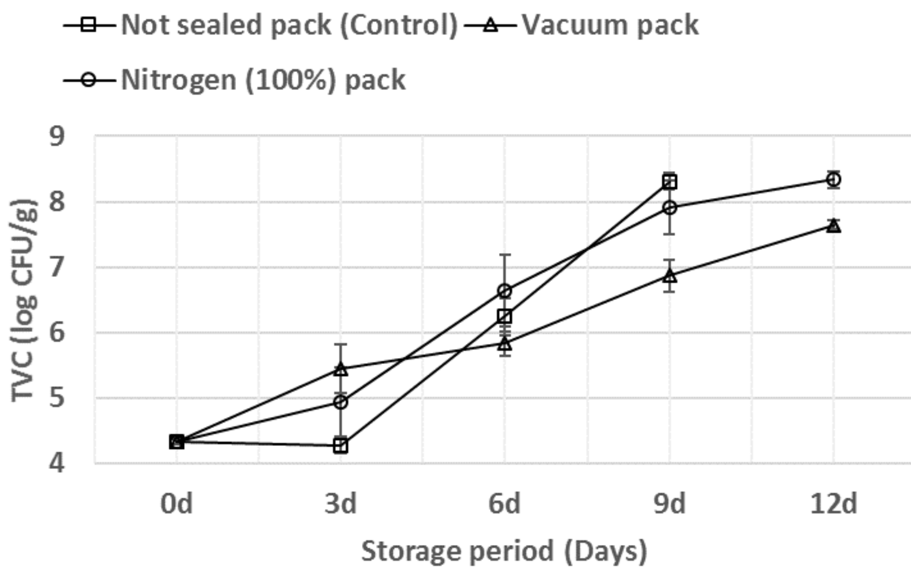

Fig. 1. Changes in the total viable count (log CFU/g) of sliced pangasius fishes under different packaging conditions at refrigerated storage

There is an agreement with several studies including Davis (2003) who found higher TVC on aerobic, not sealed pack sample compared to MAP samples which exceed the limit after 10 days of storage. Reddy *et al.* (1995) reported a higher shelf-life of MA-packaged (75% CO_2/25 N_2) tilapia fillets stored at 4°C that is of >25 days based on microbial evaluation which is longer than the finding from this study. This is due to the use of a high amount of carbon dioxide along with nitrogen. Therefore, only nitrogen flushing is not enough to increase product shelf-life. In the present study, the best performance was observed for vacuum packaging, as the slightly lower bacterial count was observed during the storage period compared to other packaging conditions. Vacuum packaging creates anaerobic conditions inside the pack; as a result, aerobic bacterial growth is inhibited, oxidative rancidity is retarded, and subsequently, the shelf-life is enhanced.

Conclusions

Vacuum and modified atmosphere packaging are getting popular with the consumer due to providing quality fishery products. It can also ensure other quality attributes, such as stabilizing the colour of the product and prevent objectionable odour development. The application of vacuum and modified atmosphere packaging in combination with chilling storage can delay or inhibit the microbial activities and increase the shelf-life of fish and fishery products. By evaluating all the biochemical and microbiological analyses, it can be concluded that vacuum packaging technology extends the shelf-life and improve the overall quality of pangasius fish compared to other packaging conditions. High production cost, the requirement of skilled staff, chemical and microbiological hazards in raw fish are some of the limitations seeming significant for developing MAP technology. Quality packaging material with various optimized combination of gas mixtures and hazard-free fresh fish has prospects for developing MAP technology in Bangladesh. Therefore, the superstores of the country can adopt this packaging technology to supply the quality fish and fishery products for the consumers.

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