Extension of shelf-life of ready-to-cook (RTC) pangas fish (Pangasianodon hypophthalmus) curry by modified atmosphere packaging at chilled storage

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Abstract. Ready-to-cook (RTC) fish products are getting popular due to busy lifestyle of the people. However, preservation is a big concern for these types of perishable food items. Modified atmosphere packaging (MAP) is a widely used packaging technique used for displaying chilled fish and fishery products in developed countries. In light of this, the quality and shelf-life of RTC pangas fish (Pangasianodon hypophthalmus) curry was evaluated by biochemical and microbiological analysis under not sealed pack (control), MAP-1 (50% CO2 & 50% N2) and MAP-2 (75% CO2 & 25%N2) pack at 4 days interval during 20 days of storage at 4°C. The pH value of pangas fish curry was in the range of 6.01 to 6.80 during the storage period. The total volatile base nitrogen (TVB-N) value in fish curry gradually increased with the storage time. However, there were no significant (p<0.05) differences in pH and TVBN values among all packaging conditions during storage. Thiobarbituric acid reactive substances (TBARS) fluctuated between 0.03 to 0.56 mg malonaldehyde/kg during the storage period. The pH, TVB-N and TBARS values under all packaging conditions were within the acceptable limit during the storage period. The total viable count (TVC) gradually increased with time in all packaging conditions. However, significantly (p<0.05) lower TVC values were observed on the 12th and 16th day of storage in all samples compared to that of the control sample. Considering the bacterial counts, the shelf-life was determined at approximately 9 days for not sealed pack, 13 days for MAP-1, and 15 days for MAP-2 sample, based on the 7 logs CFU/g, which is considered as the upper acceptable limit for fresh and frozen fish. Therefore, the MAP-2 (75% CO2 & 25%N2) is the best packaging, which can be utilized by the superstores to display this kind of value-added products with extended shelf life.

Keywords: RTC; pangas; curry; MAP; shelf-life.

1. Introduction

Pangas catfish (Pangasianodon hypophthalmus) has become one of the most popular commercial cultivable aquaculture fish species due to its high production within a short period. Total pangas production in Bangladesh was 0.51 million tons in 2016-17 [1]. This species has great economic importance because of its high flesh content which composed of thick layer of fat and good amount of protein [2]. Moreover, it is available all over the year due its higher survival rate and yield. Thus poor people can afford this fish even with their low income. The production cost was low and possessed high demand for both rural and urban people in Bangladesh [3]. Nowadays, the market price becomes very low compared to the production cost, which makes the farmer discourage to culture this fish in...
Bangladesh. Development of different value-added products from pangas fish may act as an alternative way to increase the value of fish [4].

On the other hand, there is a high demand for low cost and nutrient-rich food particularly in developing countries [5]. The city-dwellers particularly busy mothers and housewives seek ready-to-cook (RTC) foods to save their time and extra burden. The demand for ready-to-cook (RTC) or ready-to-eat (RTE) food is rising because of modern lifestyle. RTC products are developed by food companies to meet the requirement of easy preparation [6]. There is a great demand for value-added fish products, either RTC or RTE due to recent social and cultural change [7]. In Bangladesh, presently about 30 companies with more than 200 outlets of superstores have been operating in the Bangladesh which is playing a significant role in supplying good quality fish and fishery products in the country [8].

In Bangladesh, consumers usually buy the fish as raw, and most of the time the sellers do not maintain proper storage condition. As a result, a considerable amount of raw fishes confronted quality deterioration during marketing [9]. Value-added fish products could be whole, mince based products, battered & breaded products and surimi based products. Fish mince is versatile and can be used to make a variety of products such as fish fingers, fish cakes and fish sausage [10]. Fish sticks are very popular ready-to-cook value-added product. It is preferable for its characteristics flavor, firmness and test [11]. Surimi is another value-added product enriched with concentrated myofibrillar protein [12]. Surimi is the raw material for gel-based snack foods such as kamaboko and fish balls [13]. Tempura and Chikwa (Japanese surimi based products), fish sausage, fish burger are some popular value-added fish products. Fish curry can be excellent value-added products for consumers in our country as their food habits to have curry daily in their meal.

Fish and fishery products are well known to the source of nutrients due to their high protein content [14]. Fresh fish muscle is highly perishable product with a short shelf life due to the presence of unsaturated fatty acids [15]. Spoilage is the undesirable changes that make the food unsafe for human consumption. The food is no longer usable, and complete degradation takes place [16]. The spoilage of fish or fishery product is caused by enzymatic, bacterial and chemical action [17]. Chilling is the most common short term preservation technique done by lowering the temperature [18]. The purpose of chilling is to prolong the shelf-life of fish by reducing the spoilage process. Beside all these preservation technique packaging technologies has been improved a lot in the developed countries. To fulfill the high demand for fresh and convenient foods without introduction of chemical preservatives, modified atmosphere packaging (MAP) can be a preferred packaging method.

MAP is a preservation method by altering the atmospheric environment around a perishable food with a single or a mixture of protective gas [19]. MAP is a widely used packaging technique for chilled fish and fishery products in developed countries. It extends the shelf-life at refrigerated temperature [20]. In recent years, consumer demand has been increased for ready-to-cook fish products. MAP along with refrigerated storage, has become popular preservation techniques. MAP also brought significant changes in storage, distribution and marketing of raw and processed fishery product [21].

To maintain increased shelf life and keeping quality, modified atmosphere packaging (MAP) offers technological options to the fishery product [22]. MAP lengthening the shelf life of a product and reduce economic losses. It also ensures to supply a better quality product [23]. This technology allows delivering the product to long-distance markets; therefore commercial value may increase [24]. This type of packaging system has not yet developed in Bangladesh. The overall goal of this research is to increase the value and shelf-life of ready-to-cook fish and fishery products in the country and develop a proper MAP packaging system under refrigerated condition. The specific objective is to develop pangas fish curry as a ready-to-cook (RTC) fish product and determine the overall quality and shelf-life of the product under modified atmosphere packaging stored at 4°C.
2. Materials and Methods

2.1. Sample Collection and Preparation of RTC Fish Curry
The pangas catfishes (*Pangasianodon hypophthalmus*) weighting 1.5±0.3 kg were purchased from the local market and brought under live condition to the Quality Control Lab of Department of Fisheries, University of Rajshahi, Bangladesh. Upon arrival, they were washed with tap water to remove contaminants from the skin and then beheaded, gutted and cut into slices at approximately 70 g using sharp knife. Slices were washed two times with tap water, and the final wash was done with distilled water. For preparation of RTC pangas fish curry from one kilogram of fish, different ingredients and spices including 1½ teaspoon of ginger paste; 1 teaspoon of garlic paste, red chili powder, coriander powder, salt; ½ teaspoon of cumin powder, lemon juice and ¾ teaspoon of turmeric powder were required. All the ingredients were taken in a bowl and added little amount of water to make a smooth paste. Then the slices were mixed with paste properly in the bowl and kept at refrigerator for twenty minutes after wrapping with plastic.

2.2. Packaging and Storage of RTC Fish Curry
The required amount of RTC fish curry was packed under modified atmosphere packaging in low gas and moisture permeable plastic pouch. The packaging material used for this purpose was multilayer transparent pouch (PE/PA/PE) of having 100 μm densities. Three types of packaging were applied under modified atmosphere packaging with different gas ratio using the method described by Noseda et al. [25]. MAP packaging was performed using a packaging machine (C100 Multivac, Haggenmuller, Germany) attached with Gas Mixer (KM100-3 MEM, WITT, Germany) by following the manual instruction of the machine. Analysis of the O₂, N₂ and CO₂ levels in the headspace of the packaged samples were performed with a gas analyzer (Oxybaby M+, WITT, Germany). Those three types of packaging was used as treatments namely, (1) Not sealed pack as treatment-0 (control); (2) MAP-1 (50% CO₂ & 50% N₂) as treatment-1 and (3) MAP-2 (75% CO₂ & 25% O₂) as treatment-2. All samples were stored at 4°C in the laboratory refrigerator. Three samples from each packaging condition were analyzed at four days interval during the storage at 4°C for 20 days in the laboratory.

2.3. Biochemical and Microbiological Analysis
The biochemical and microbiological parameters were analyzed in the laboratory to know the quality of RTC fish curry as well as to determine the shelf-life of curry under chilled storage. The pH value of the fish flesh homogenate was measured by means of a glass electrode pH meter (HI2002-Edge, Hanna Inst, USA) using the instruction manual. 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 [26] method. Thiobarbituric acid reactive substances (TBARS) values were measured by colorimetric method using the procedure of Witte et al. [27]. TBARS values was calculated as follows: TBARS value (mg malonaldehyde/kg) = optical density (O.D.)×5.2. Total viable count (TVC) was determined by a standard plate count method on plate count agar by APHA [28] method. Plates were incubated at 35°C in an incubator for 48 hours and counted the colony.

2.4. Statistical Analysis
The values were expressed as mean ± standard deviation. Differences among treatments were estimated by using one-way ANOVA with the application of the Tukey test using SPSS Version 20. Average values were considered significantly different when p<0.05.

3. Result and Discussion
The present study was undertaken to assess the shelf life of ready-to-cook (RTC) pangas (*Pangasianodon hypophthalmus*) fish curry under modified atmosphere packaging at 4°C. The quality and shelf life of the pangas fish curry was determined by evaluating various biochemical parameters namely pH, total volatile base nitrogen (TVB-N), thiobarbituric acid reactive substance (TBARS) value and microbiological analysis through total viable count during 20 days of storage period.
3.1. pH Value

One of the most important indicators to determine the quality of fish and fishery products is pH. Muscle pH can be used as a biochemical method to assess the freshness of fish. The acceptable limit of postmortem pH ranges between 6.8–7.0 [29]. At the beginning of the study, pangas fish curry showed a pH value of 6.69. Then the pH value was in decreasing trend until the 8th day of storage for MAP-1 (50% CO₂ & 50% N₂) and MAP-2 (75% CO₂ & 25% N₂) samples and 4th day of storage for control sample, and after that an increasing trend was observed at rest of the storage period (Table 1). However, there was no significant (p<0.05) difference in pH values among three packaging conditions during the storage period. Besides, pH values were within the acceptable limit in all packaging conditions throughout the storage period.

**Table 1.** pH value of RTC pangas fish curry under three different packaging conditions at chilled storage (4°C).

| Treatments                  | Storage period (days) |
|-----------------------------|-----------------------|
|                             | 0 d  | 4 d  | 8 d  | 12 d | 16 d | 20 d |
| Not sealed pack (Control)   | 6.69±0.06a | 6.32±0.10a | 6.62±0.18a | 6.64±0.16a | 6.77±0.17a |
| MAP-1 (50% CO₂ & 50% N₂)    | 6.69±0.06a | 6.80±0.02a | 6.22±0.37a | 6.56±0.08a | 6.21±0.07a |
| MAP-2 (75% CO₂ & 25% N₂)    | 6.69±0.06a | 6.47±0.27a | 6.01±0.00a | 6.27±0.20a | 6.39±0.20a |

This declined trend of pH perhaps happened due to the surface reaction of CO₂ with water forming carbonic acid, which results in the acidification of the fillets [30]. Another reason might be due to the accumulation of lactic acid by anaerobic glycolysis and the liberation of inorganic phosphates by the degradation of ATP. This is an agreement with the result reported by Ayala et al. [31] for sea bream during 22 days of ice storage. Islami et al. [32] also reported that sardine (Sardina pilchardus) fish showed a sharp decrease of pH after their marination with different concentrations of acetic acid and salt solutions. On the other hand, the increasing trend of pH at later stages of storage may be happened due to the production of amines and other volatile bases by the autolytic and microbial action on protein and other components [33, 34]. Similar increasing behavior was observed during storage in brined chub mackerel [35], marinated anchovies [36] at refrigerated temperature.

3.2. Total Volatile Base Nitrogen (TVB-N) Value

Chemical spoilage of fish samples can be evaluated by measuring the changes of total volatile base nitrogen (TVB-N) content which mainly comprises ammonia (NH₃), dimethylamine (DMA) and trimethylamine (TMA) [37] and is commonly used as an estimation of spoilage and has been widely used as an index for freshness of fish [38]. The acceptable limit of TVB-N value for freshly caught fish is 5.6 mgN/100g, and ice-stored cold-water fish is 30–35mgN/100g of fish [39].

**Table 2.** TVB-N value (mg/100g) of RTC pangas fish curry under three different packaging conditions at chilled storage (4°C).

| Treatments                  | Storage period (days) |
|-----------------------------|-----------------------|
|                             | 0 d  | 4 d  | 8 d  | 12 d | 16 d | 20 d |
| Not sealed pack (Control)   | 3.36±0.40a | 3.36±0.00a | 4.20±0.40a | 5.88±0.40a | 6.75±0.16a |
| MAP-1 (50% CO₂ & 50% N₂)    | 3.36±0.40a | 3.72±0.68a | 4.00±0.68a | 6.02±0.59a | 6.42±0.54a |
| MAP-2 (75% CO₂ & 25% N₂)    | 3.36±0.40a | 3.50±0.76a | 5.18±0.20a | 5.65±1.06a | 6.22±0.30a | 7.49±0.21a |

Different superscript letters in the same column represent a significant difference among the means of treatments (p < 0.05).

In the present study, the initial TVB-N value of RTC pangas fish curry was 3.36 mg/100 g and then gradually increased with time during the storage period in all packaging conditions (Table 2). There were no significant (p>0.05) differences found in relation to TVB-N values among three packaging conditions during the storage period. However, the TVB-N values were within the acceptable limit...
(30-35 mg/100g) in all packaging conditions (Table 2). Similar results were observed by Soccol et al. [40] that there were no significant differences on TVB-N values among the treatments of tilapia fish (air, vacuum and MAP with 60% CO_2/40% O_2) during the 20 days of storage period 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. Sivertsvik et al. [41] reported that TVB-N increased on MAP-1 (CO_2/N_2: 80/20) & MAP-2 (CO_2/N_2: 60/40) packaging system of filleted Cod fish (Gadus morhua) during 28 days of storage where MAP-1 showed higher TVB-N compared to MAP-2. In their study, they also reported no significant difference (p > 0.05) of TVB-N values during 90 days of storage [35]. A previous study conducted by Noseda et al. [25] also reported that TVB-N values were increased for Vietnamese pangas (Pangasius hypophthalmus) with various gas mixture (50%CO_2/50%N_2; 50%CO_2/50%O_2) during 60 days of storage.

3.3. TBARS (Thiobarbituric Acid Reactive Substance) value

TBARS (Thiobarbituric acid reactive substance) value is a widely used indicator for the assessment of the degree of lipid oxidation. It is mainly the measure of secondary lipid oxidation. The acceptable limit of TBARS value is 2 mg malonaldehyde/kg fish. However beyond this limit, an objectionable odor and taste develop in fish [42].

In the present study, the initial TBARS value was 0.14 mg malonaldehyde/kg RTC pangas fish curry. The TBARS value fluctuated between approximately 0.1 to 0.2 mg malonaldehyde/kg until the 12th day of storage in all packaging conditions and then gradually increased until end of the storage period (Table 3). Significantly (p<0.05) lower TBARS values were observed in all samples on 12th and 16th day of storage compared to that of control sample. TBARS values were within the acceptable limit (2 mg malonaldehyde/kg) in all samples during the storage period.

**Table 3.** TBARS value (mg malonaldehyde/kg) of RTC pangas fish curry under three different packaging conditions at chilled storage (4°C).

| Treatments                  | 0 d     | 4 d     | 8 d     | 12 d    | 16 d    | 20 d     |
|-----------------------------|---------|---------|---------|---------|---------|---------|
| Not sealed pack (Control)   | 0.14±0.10a | 0.18±0.08b | 0.18±0.05a | 0.16±0.01b | 0.39±0.01b |         |
| MAP-1 (50% CO_2 & 50% N_2) | 0.14±0.10a | 0.12±0.03a | 0.08±0.04a | 0.14±0.00ab | 0.29±0.00a |         |
| MAP-2 (75% CO_2 & 25% N_2) | 0.14±0.10a | 0.24±0.07a | 0.11±0.09a | 0.13±0.01a | 0.29±0.03a | 0.56±0.12 |

Different superscript letters in the same column represent a significant difference among the means of treatments (p < 0.05).

There is an agreement with the findings of Sallam et al. [43] showed an initial TBARS value of 0.37 mg malonaldehyde/kg in fresh raw Pacific saury where lower values observed in not sealed aerobic pack sample compared to that of MAP (75% CO_2 & 50% N_2 & 50% CO_2 & 75% N_2) sample. In the present study, MAP-2 sample having 75% CO_2 showed higher TBARS value than MAP-1. Ruiz-capillas and Moral [44] observed higher TBARS amounts for atmospheres richer in CO_2. This was probably due to a synergistic action between CO_2 and O_2, which made the auto-oxidation of polyunsaturated fatty acids easier. According to Aubourg [45], TBARS records may not reveal the actual rate of lipid oxidation as malonaldehyde may interact with other components of fish muscle. Such components may be 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.

3.4. Total Viable Count (TVC)

Microbiological assessment is an excellent way to evaluate product quality. One of the most common methods of microbial assessment is the determination of bacterial count [46]. The initial total viable count (TVC) of sliced tilapia fishes was 5.11 Log CFU/g on plate count agar medium that indicated an acceptable initial quality of fish. Most of the available literature on freshly caught freshwater fishes (sea bass, tilapia, rainbow trout, and silver perch) reported bacterial counts of 2-6 Log CFU/g [47].
Table 4. Total viable count (log CFU/g) of pangas fish curry under three different packaging conditions at chilled storage (4°C).

| Treatments                  | Storage period (days) | 0 d   | 4 d   | 8 d   | 12 d  | 16 d  | 20 d  |
|-----------------------------|-----------------------|-------|-------|-------|-------|-------|-------|
| Not sealed pack (Control)   |                       | 5.11±0.33<sup>a</sup> | 5.21±0.40<sup>a</sup> | 6.65±0.30<sup>a</sup> | 7.90±0.13<sup>b</sup> | 9.25±0.48<sup>b</sup> |
| MAP-1 (50% CO<sub>2</sub> & 50% N<sub>2</sub>)|                       | 5.11±0.33<sup>a</sup> | 5.17±0.42<sup>a</sup> | 6.12±0.35<sup>a</sup> | 6.74±0.16<sup>a</sup> | 7.85±0.09<sup>a</sup> |
| MAP-2 (75% CO<sub>2</sub> & 25% N<sub>2</sub>)|                       | 5.11±0.33<sup>a</sup> | 4.92±0.10<sup>a</sup> | 5.85±0.55<sup>a</sup> | 6.39±0.22<sup>a</sup> | 7.21±0.31<sup>a</sup> | 7.71±0.27<sup>a</sup> |

Different superscript letters in the same column represent a significant difference among the means of treatments (p < 0.05).

In the present study the TVC gradually increased with the progression of storage time in all packaging conditions. There was no significant difference in TVC observed among all packaging conditions until the 8<sup>th</sup> day of storage. However, significantly (p<0.05) lower TVC were observed on 12<sup>th</sup> and 16<sup>th</sup> day of storage in all packaged samples compared to that of control sample (Table 4).

![Figure 1](image-url) Total viable count (log CFU/g) of pangas fish curry under three different packaging conditions at chilled storage (4°C).

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 [48] on approximately 9<sup>th</sup> day for not sealed pack (control), 13<sup>th</sup> day for MAP-1 and 15<sup>th</sup> day of storage for MAP-2 sample (Figure 1). Taking the 7 Log CFU/g as the maximum acceptable limit for fresh, frozen and cold-smoked fish species, the shelf-life of RTC pangas fish curry was determined at approximately 9 days for not sealed pack, 13 days for MAP-1 (50% CO<sub>2</sub> & 50% N<sub>2</sub>), and 15 days for MAP-2 (75% CO<sub>2</sub> & 25% N<sub>2</sub>) sample.

There is an agreement with several studies including Davis <i>et al.</i> [49] who found higher total viable count on aerobic, not sealed pack sample compared to MAP samples which exceed the limit after 10 days of storage. He also observed lower bacterial counts in MAP (70%CO<sub>2</sub> & 30%N<sub>2</sub>) compared to MAP (30%CO<sub>2</sub> & 70%N<sub>2</sub>) sample of Cod fillet. In the present study, MAP-2 contain more CO<sub>2</sub> (75%) than MAP-1 (50%) which is the main factor. Fagan <i>et al.</i> (2004) also observed higher total viable count on Salmon fish under MAP (60% N<sub>2</sub>/40% CO<sub>2</sub>), which contains less CO<sub>2</sub> concentration compared to MAP (100%CO<sub>2</sub>). They also reported that high CO<sub>2</sub> level delayed microbial growth.

In the present study, the best performance was observed in case of MAP-2 (75% CO<sub>2</sub> & 25%N<sub>2</sub>) packaging. In addition to this system, lower bacterial count was observed during the storage period compared to other packaging condition. Perhaps this type of packaging created anaerobic condition.
inside the pack; therefore, it’s inhibited the aerobic bacterial growth and increased the shelf-life of the product. Besides, CO₂ possess bacteriostatic effect, which also retards the oxidative rancidity and inhibits the growth of aerobic microorganisms from the system [50].

4. Conclusions
Modified atmosphere packaging is getting popular to the consumer due to providing excellent quality fishery products. MAP system can also ensure other quality attributes such as stabilizing color of the product and prevent objectionable odor. The shelf life of fishery products can be hampered by MAP technology in our country. Different value added products need to be optimized by this system which will ensure better quality fish and fishery products with an extended shelf life.

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