

# Does Vacuum Packaging Retain the Quality of Hilsa Fish (*Tenualosa ilisha*) during Long-Term Frozen Storage? †

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**Abstract:** The long-term frozen storage deteriorates the quality of hilsa fish (*Tenualosa ilisha*), famous for its delicious taste and flavors. Vacuum packaging may retain the flavor and quality of hilsa fish. To this end, biochemical and microbiological analyses were used to assess the quality of hilsa fishes under whole without pack, whole with vacuum pack, dressed without pack, and dressed with vacuum pack at a monthly interval during a year of frozen storage at  $-18\text{ }^{\circ}\text{C}$ . There were no such significant differences ( $p > 0.05$ ) in the values of pH, free fatty acids (FFA), total volatile base nitrogen (TVB-N), and aerobic plate count (APC) among the treatments, and the values remained within the acceptable limit during the storage period. However, thiobarbituric acid reactive substances (TBARS) were significantly ( $p < 0.05$ ) lower in samples under vacuum (whole & dressed) compared to samples without pack. TBARS values did not cross the acceptable limit for vacuum pack samples in nearly the entire storage period but exceeded for samples without pack within the first month. It is assumed that the vacuum packaging reduced the secondary oxidation of fatty fish like hilsa during the frozen storage, which can ensure a year-round supply of quality hilsa fish without altering the distinctive taste and flavor.

**Keywords:** hilsa fish; quality; vacuum packaging; rancid odor

## 1. Introduction

Hilsa fish (*Tenualosa ilisha*), an anadromous fish migrating from the sea for spawning in freshwater rivers, is an important fishery in some Asian and Middle East countries [1]. Hilsa is Bangladesh's national fish and is highly famous for its delicious taste and unique flavors, and high socio-economic importance [2,3]. It is economically the most significant single species, employing about 0.45 million people directly, and about 2.5 million people indirectly ([2]. Globally, 86% of hilsa come from Bangladesh, followed by India (8%), Myanmar (4%), and the rest from other countries [3]. Total hilsa production was 0.52 million MT in 2017–2018, sharing 12.09% of the total fish production in Bangladesh. About 60–70% of this high demanded fish is consumed fresh in Bangladesh, and the rest is exported to India, the USA, the EU, Japan, and the Middle-east [4].

In hilsa fishing, two types of boats are used; a larger boat having icing facilities is engaged fishing for few days to a month, and a small boat without icing facilities is engaged fishing near the shore for

few hours to a whole day and iced fish on-shore. As a result, improper onboard icing and without icing, hilsa shad undergoes deterioration during this period. On the shore, hilsa fishes are iced in different containers, mostly in Styrofoam box and transported in many ways [5,6].

Hilsa fishes are traded in domestic markets as fresh, whole, and without dressing and adding ice. High fat content makes the hilsa fish highly susceptible to oxidative rancidity, along with rapid autolysis and bacteriological decomposition [6]. Therefore, careful handling is necessary at all stages of transportation, processing, preservation, and marketing to control and slow down spoilage [7].

Hilsa cannot be sun-dried due to its high lipid content. Therefore, icing is the most common short-term preservation process, and salting or salt-fermenting is the long term one. Freezing and frozen storage are also sometimes practiced [8]. However, this long-term frozen storage deteriorates hilsa quality due to the oxidation of lipid and myoglobin's disintegration along the lateral line [9]. Any intervention with freezing is required to retain the quality and flavor for a more extended period. Vacuum packaging, a proven packaging method, could be an effective intervention along with freezing to increase shelf life, which will ensure a year-round supply of quality fish in the market.

## 2. Materials and Methods

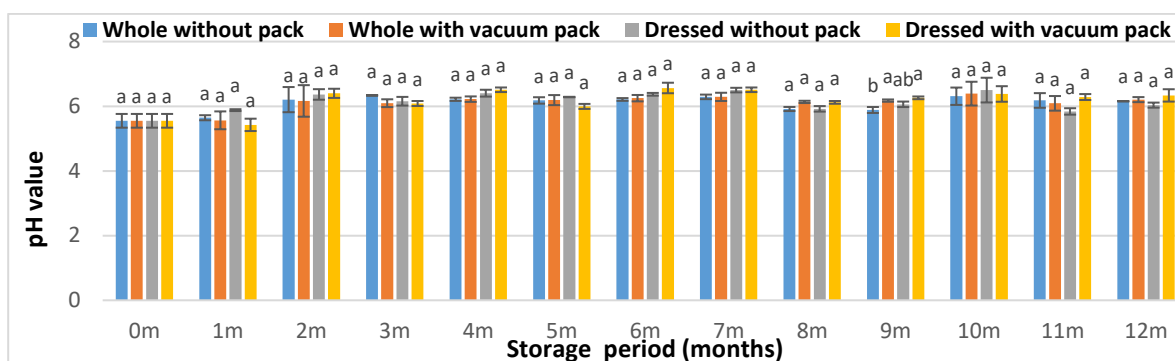
**Sample collection and preparation:** Hilsa fishes weighing  $0.8 \pm 0.15$  kg were bought from the hilsa landing center in Chandpur, Bangladesh. The fishes were then brought to the Quality Control Laboratory of the Department of Fisheries at the University of Rajshahi under icing condition. After arriving, half of the fishes were dressed, and half were kept as a whole. In both cases, fishes were washed properly with tap water. Four types of packaging were used as treatments, namely; (a) whole without pack as control, (b) whole with vacuum pack; (c) dressed without pack; and (d) dressed with vacuum pack. Vacuum packaging was done in low oxygen and moisture permeable plastic pouch (PE/PA/PE) using Multivac C100 packaging unit (Haggenmueller, Germany). A total of 132 fishes (33 in each treatment) was kept separately in frozen storage at  $-18$  °C for 12 months. The fishes in triplicate were subjected to biochemical and microbiological analysis at 30 days interval in the laboratory.

**Biochemical and Microbiological Analysis:** The pH of the hilsa fish was determined by a pH meter (HI2002, Hanna Inst, USA) [10]. Total volatile base nitrogen (TVB-N) was measured using EC method [11]. Thiobarbituric acid reactive substance (TBARS) was estimated using a colorimetric method [12]. Total viable count (TVC) was determined on plate count agar (Sigma-Aldrich, USA) by standard pour plate method [13] and expressed as colony-forming units (CFU/g). Plates were kept for 48 h in an incubator (Poleko, Poland) at 35 °C and later colonies were counted.

## 3. Results

### 3.1. pH Value

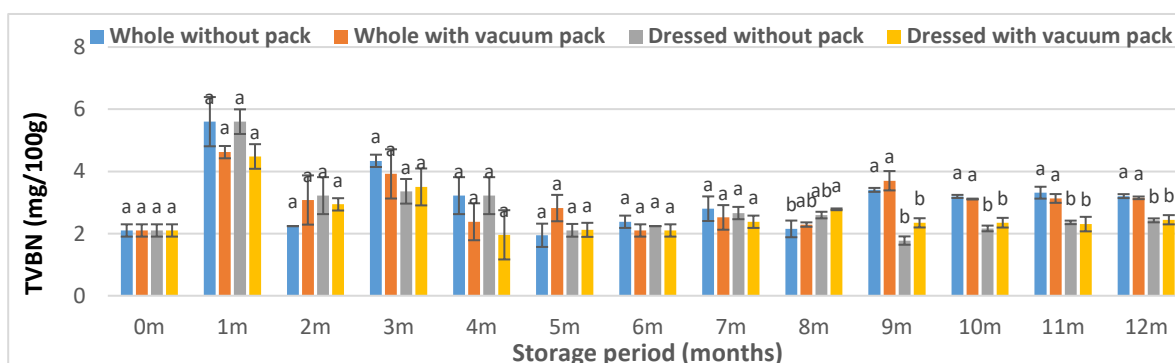
In this study, the initial pH of hilsa fish was 5.55 and then gradually increased to the 2nd month of the storage. The pH values were then fluctuated from 5.84 to 6.50 for samples without pack and 6.00 to 6.57 for vacuum pack fish samples (Figure 1). However, no significant differences ( $p > 0.05$ ) were observed among the treatments in the entire storage period.



**Figure 1.** Changes in pH value of hilsa fish under vacuum packaging conditions during frozen storage. Different letters on the top of each month’s bars denote a significant difference among the means of treatments ( $p < 0.05$ ).

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

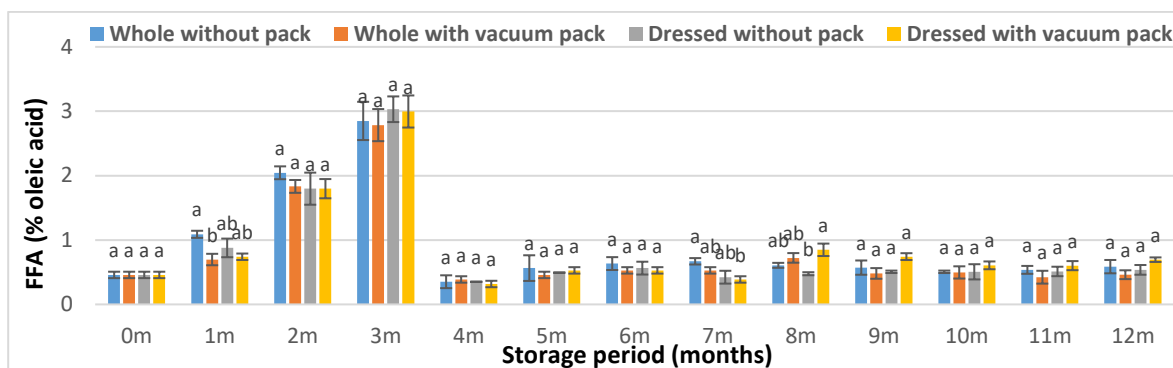
The initial TVB-N value was 2.1 mg/100 g and then increased sharply in the 1st month, and later a decreasing trend with fluctuations was observed until the 4th month for the vacuum pack samples and the 5th month for samples without pack. After that, the TVB-N values in all samples fluctuated in the rest of the storage period. The ranges of TVB-N value was 1.8 to 5.6 mg/100 g. There was no such significant difference ( $p > 0.05$ ) among the treatments until the 8th month of storage. However, significantly ( $p < 0.05$ ) lower TVB-N values were observed in dressed fishes than whole fishes on 9th month and onwards.



**Figure 2.** Changes in TVB-N value (mg/100 g) of hilsa fish under vacuum packaging conditions during frozen storage. Different letters on the top of each month’s bars denote a significant difference among the means of treatments ( $p < 0.05$ ).

### 3.3. Free Fatty Acids (FFA)

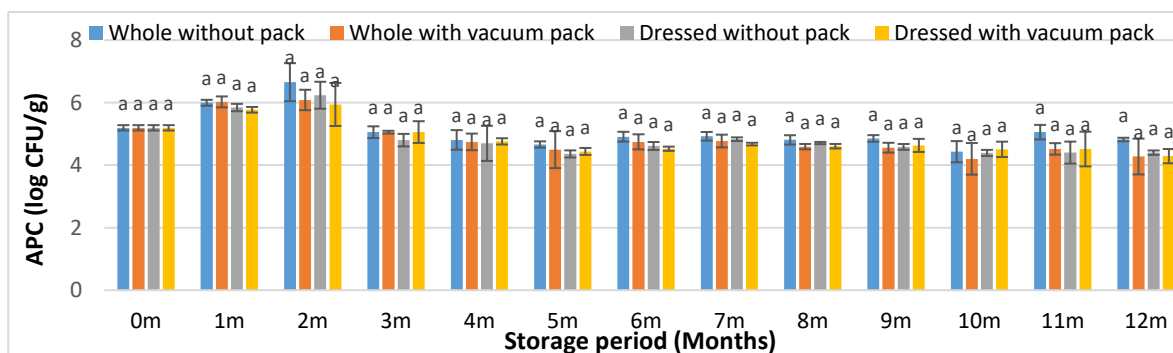
The initial FFA value of hilsa fish was 0.46% oleic acid and then gradually sharply increased up to 2.78–3.03% in the 3rd month and then sharply decreased to 0.32–0.39% on the 4th month and kept almost steady in rest of the storage (Figure 3). However, there was no such significant difference ( $p > 0.05$ ) among the treatments during the entire storage.



**Figure 3.** Changes in FFA value (% oleic acid) of hilsa fish under vacuum packaging conditions during frozen storage. Different letters on the top of each month’s bars denote a significant difference among the means of treatments ( $p < 0.05$ ).

### 3.4. Aerobic Plate Count (APC)

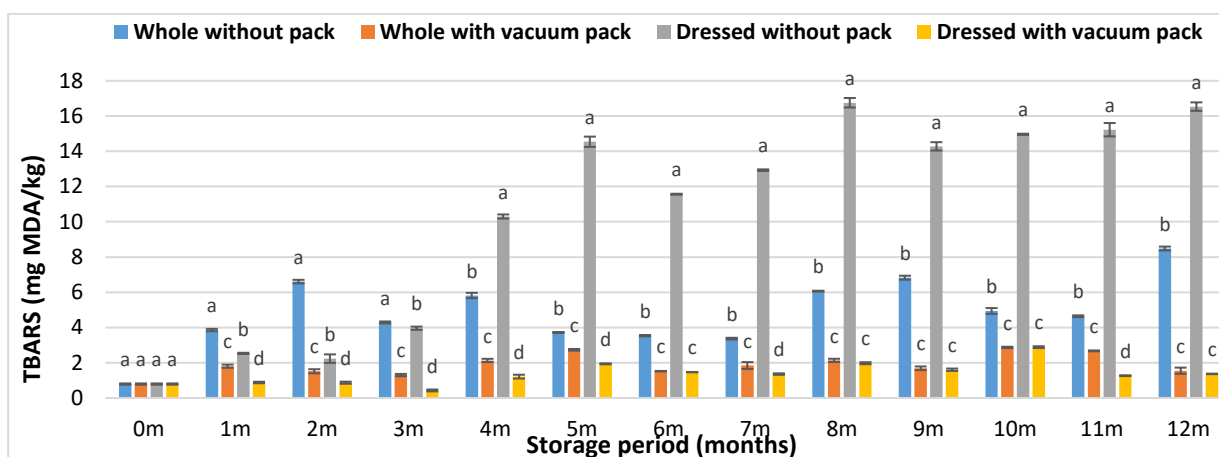
The aerobic plate count (APC) of fresh hilsa fish was 5.19 log CFU/g indicated an acceptable initial quality of hilsa fish. The APC values of hilsa fish gradually increased until the 2nd month and then gradually decreased to around 4.5 log CFU/g in the 5th month and remained steady with some fluctuations in the rest of the storage period (Figure 4). However, no significant difference ( $p > 0.05$ ) was observed among the treatments during the storage period.



**Figure 4.** Changes in aerobic plate count (log CFU/g) of hilsa fish under vacuum packaging condition during frozen storage. Different letters on the top of each month’s bars denote a significant difference among the means of treatments ( $p < 0.05$ ).

### 3.5. Thiobarbituric Acid Reactive Substance (TBARS) Value

The initial value of TBARS was 0.79 mg malondialdehyde (MDA)/kg of hilsa fish. The TBARS amount gradually increased in the whole fish sample without pack up to the 2nd month and then slowly decreased until the 7th month, and then an increasing trend was observed (Figure 5). In the case of dressed without pack samples, the TBARS value gradually increased up to the 3rd month and then sharply increased to 14.54 mg MDA/kg on the 5th month and later increased slowly with some fluctuations in the rest of the storage period. For vacuum pack samples (whole and dressed), the TBARS amounts fluctuated almost within the acceptable limit in the entire storage period (Figure 5).



**Figure 5.** Changes in TBARS value (mg malondialdehyde/kg) of hilsa fish under vacuum packaging conditions during frozen storage. Different letters on the top of each month's bars denote a significant difference among the means of treatments ( $p < 0.05$ ).

However, TBARS amounts were significantly ( $p < 0.05$ ) affected by the vacuum packaging in both whole and dressed fish during the storage period. Significantly ( $p < 0.05$ ) lower TBARS amounts were found in vacuum pack samples (whole and dressed) compared to the samples without pack in the entire storage period. Besides, significantly ( $p < 0.05$ ) lower TBARS amounts were found in dressed with vacuum pack samples compared to the whole with vacuum pack in most sampling days. In the case of vacuum pack samples, significantly ( $p < 0.05$ ) lower TBARS amounts were observed in whole fish compared to that of dressed fish on the 4th month and onwards.

#### 4. Discussion

pH is an indicator factor for the quality evaluation of fish and fishery products. The appropriate post mortem pH level of fish is typically 6.8–7.0 [14]. pH values of all samples were within the acceptable limit during the storage period. Increased pH up to two months could be due to the accumulation of amines and other volatile compounds through the autolytic and microbial activity on proteins and other compounds [15].

TVB-N content is commonly used as an indicator for predicting bacterial spoilage of fish [16]. The TVB-N values of 30–35 mg N/100 g fish is generally considered an acceptability limit for fresh and ice-stored fish [17]. In the present study, the increasing TVB-N value in the 1st month could be due to the production of basic compounds via microbiological action on protein and non-protein nitrogenous compounds. This initial TVB-N rise accords with the increase in pH up to 2nd month in the present study. However, the TVB-N contents of all samples were within the acceptable limit.

FFA is considered to have adverse effects on protein properties; thus, FFA accumulation has been associated with a lack of acceptability and oxidizes faster than higher molecular weight lipid groups [18]. FFA increased during the frozen storage, suggesting that lipid hydrolysis occurred at low temperatures as well (Figure 3). This rise was found to be noticeably higher during the first stage of the experiment (months 0–3 at  $-18\text{ }^{\circ}\text{C}$ ), which is in line with previous research [19,20]. This activity has been explained by a maximum lipase release from liposomes during storage, which allows closer proximity between enzyme and substrate [21]. However, after the initial increase, a similar decreasing trend was observed after 3rd month for frozen white cheek shark in frozen storage [22]. This decrease may be attributed to the depletion of substrate and oxidation of FFA [23]. In this study, the FFA content of hilsa fishes was within the acceptable limit (5% oleic acid) in entire storage [24].

The bacteria counts of 2–6 Log CFU / g are considered the satisfactory initial standard of freshly caught freshwater fish [25], which indicated the acceptable initial quality of the hilsa fish. In a previous study, the total bacterial count of 5.47 log CFU/g was observed in frozen hilsa fish after one month of frozen storage, which is slightly lower than that of the present study [26]. In this study, the

APC values did not exceed the 7 log CFU/g [27], considered to be upper acceptable limit for fresh and frozen fish in all samples over the storage period (Figure 4).

The thiobarbituric acid reactive substances (TBARS) number is a measure of malondialdehyde (MDA), a by-product of secondary lipid oxidation [28]. The 2 mg MDA/kg fish is the acceptable limit of TBARS value, and when this limit exceeds, an obnoxious odour and taste build up in fish [29]. In this study, a gradual increase of TBARS value with storage time was observed in samples without pack. The TBARS values crossed the acceptable limit (2 mg MDA/kg) in both whole and dressed samples under without pack samples on the 1st months and onwards (Figure 4). A similar increasing trend was observed in narrow barred Spanish mackerel fish, indicating high lipid oxidation [22]. However, in vacuum pack samples, the values were almost within the acceptable limit in the entire storage period (Figure 5). It assumed that the vacuum packaging could reduce the secondary oxidation of fatty fish like hilsa during the storage period, responsible for the rancid odor in long-term frozen stored hilsa fish. Under frozen storage conditions, vacuum packaging resulted in less oxidative degradation than traditional packaging of frozen cobia fish fillets [30]. In another study, the removal of oxygen from the package was suggested to be more important than the use of antioxidants in delaying lipid oxidation in mackerel fish [31].

## 5. Conclusions

In the present study, the vacuum packaging effectively controlled the TBARS values in both whole and dressed hilsa fishes during the storage period. This study clearly stated that the vacuum packaging prevented the oxidative rancidity in the long term frozen hilsa fishes. Thus, this vacuum packaging technique can be used along with frozen storage by the processors or even households to make the hilsa fish available year-round without hampering the quality and taste.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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