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# **Microbiological and Chemical Quality Assessment of Six Fish Species of Bangladesh during Freeze Storage**

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**Abstract**— The following six Bangladeshi fish species, Rui (*Labeo rohita*), Catla (*Catla catla*), Tilapia (*Oreochromis mossambicus*), Pangas (*Pangasianodon hypophthalmus*), Mrigal (*Cirrhinus cirrhosus*) and Carfu (*Cyprinus carpio*) were assessed for quality changes for 30 days of freezing (-10°C) storage. The bacteriological quality, chemical changes of fish fats of these six species of cultured ponds and different wholesale & retail markets of Dhaka city were analysed. Microbial load was highest in local retail markets samples and were lowest in cultured ponds samples. In total, 54 samples were analysed and they differ from species to species and with environment. For all sample, TVBC ranged from  $2.75 \times 10^5$  to  $6.29 \times 10^6$  cfu/g in first days. But they raised upto  $9.96 \times 10^8$  cfu/g for local retail market samples in 30<sup>th</sup> day. We extracted fish fats of this six species and examined 5 days interval upto 30 days and found that FFA and POV increased & Iodine value decrease day by day and with environmental condition.

**Keywords**—rui, catla, tilapia, pangas, mrigal, carfu, Bangladesh

## **I. INTRODUCTION**

Fish, an integral part of the diet in Bangladesh, is a major source of animal protein to its population [1]. The present scenario of fish trade in Bangladesh presents non-satisfactory returns from the catch due to quantitative and qualitative loss. Quantitative losses include fish, which are rejected because of low commercial value while qualitative losses occur through spoilage or insect attack. Qualitative losses consist of losses in commercial value, but not in physical biomass, through loss of quality [2]. Freezing is the method for preserving fresh fish and freezing of fish, is at present one of the most effective ways for long term method of preservation. Freezing can preserve the highly perishable fish without much change in its quality for a certain length of storage period in which the deteriorative actions of micro-organism and enzymes are partially or completely arrested [3][4]. Though freezing at -10°C may prevent bacterial spoilage, other undesirable changes still occurs resulting in deterioration of the quality of fishes. Chemical tests can measure the amounts of break-down products derived from enzymatic, bacterial or oxidation activity and have also been used for the assessment of the fish quality [5].

This study designed to investigate the quality changes of freeze storage of six selected fish species using microbiological and chemical assessment.

## **II. MATERIALS AND METHODS**

Fishes were collected from 3 different cultured ponds of Comilla, 3 wholesale market of Dhaka city and 3 retail local market of Dhaka city. We used a sterile aseptic container together with ice for collecting samples to maintain the temperature. The fish were sampled immediately after being delivered to the laboratory on elapsed day 1 and after 5 days intervals up to 30 days.

### **A. Microbiological Assessments**

The total bacterial count (TBC)/ Standard plate count (SPC) of fishes were measured 5 days interval from 0 days to up to 30 days. To measure Total Viable bacterial count we used pour plate method where we incubated them at 37°C for 24 hours. Total bacterial counts were expressed in colony forming unit per gram (cfu/g).

### **B. Chemical Analysis**

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1) *Fat Extraction*: Total fat of fishes muscle were extracted using the Bligh and Dyer [6] procedure with slight modification to adjust the final proportion of chloroform: methanol: water to 2:2:1.8 (Hansen and Olley, 1963) [7]. The concentrated fat extracts were quantitatively transferred to 250 ml volumetric flask and the volume were made up to the mark with  $\text{CHCl}_3$ . Aliquots (2ml each) were evaporated to dryness to determine the fat content of the muscle. Rest of the samples were transferred to an amber coloured bottle, kept under nitrogen atmosphere in refrigerator (3-5°C) with the addition of few crystals of BHT to stop further oxidation.

2) *Chemical Assessment*: Chemical assessment like Free Fatty acid (as oleic) present, Peroxide Value, m. eq.  $\text{kg}^{-1}$  (POV), Iodine value (Hanus) of the fish fat were determine by AOCS, (1971)[8]; and IUPAC, (1979)[9].

### III. RESULTS AND DISCUSSIONS

#### A. Microbiological Assessments

We stored 54 samples of these six species of fishes in (-10°C) for 30 days. We studied -Total Viable Bacterial Counts (TVBC) of these six species of fish samples. The Study reveals that bacterial counts were higher in local retail markets fish samples and was comparatively lower in ponds fish samples.

Table 1: Bacteriological quality assessments of Rui (*Labeo rohita*) fish samples collected from ponds, wholesale market and local retail markets for 5 days interval up to 30days:

Days	Parameters	Comilla pond-1 (cfu/g)	Comilla pond-2 (cfu/g)	Comilla pond-3 (cfu/g)	Whole sale market-1 (cfu/g)	Wholesale market-2 (cfu/g)	Wholesale market-3 (cfu/g)	Local Retail market-1 (cfu/g)	Local Retail market-2 (cfu/g)	Local Retail market-3 (cfu/g)
0	*TVBC	$2.75 \times 10^5$	$3.10 \times 10^5$	$3.90 \times 10^5$	$4.60 \times 10^5$	$5.23 \times 10^5$	$4.88 \times 10^5$	$1.89 \times 10^6$	$2.78 \times 10^6$	$5.10 \times 10^6$
5	*TVBC	$3.56 \times 10^5$	$4.11 \times 10^5$	$5.98 \times 10^5$	$5.55 \times 10^5$	$6.12 \times 10^5$	$5.34 \times 10^5$	$2.45 \times 10^6$	$4.56 \times 10^6$	$6.23 \times 10^6$
10	*TVBC	$4.56 \times 10^5$	$5.22 \times 10^5$	$6.29 \times 10^5$	$6.44 \times 10^5$	$7.44 \times 10^5$	$7.37 \times 10^5$	$3.56 \times 10^6$	$5.33 \times 10^6$	$7.29 \times 10^6$
15	*TVBC	$5.32 \times 10^5$	$6.32 \times 10^5$	$7.93 \times 10^5$	$7.21 \times 10^5$	$7.99 \times 10^5$	$8.33 \times 10^5$	$4.00 \times 10^6$	$6.98 \times 10^6$	$8.34 \times 10^6$
20	*TVBC	$6.33 \times 10^5$	$7.42 \times 10^5$	$8.11 \times 10^5$	$8.29 \times 10^6$	$8.00 \times 10^6$	$9.23 \times 10^6$	$5.36 \times 10^7$	$7.23 \times 10^7$	$8.87 \times 10^7$
25	*TVBC	$7.12 \times 10^7$	$8.32 \times 10^7$	$9.36 \times 10^7$	$9.54 \times 10^7$	$9.36 \times 10^7$	$9.36 \times 10^7$	$6.69 \times 10^8$	$8.55 \times 10^8$	$9.32 \times 10^8$
30	*TVBC	$8.36 \times 10^7$	$9.23 \times 10^7$	$9.38 \times 10^7$	$9.55 \times 10^8$	$9.49 \times 10^8$	$9.50 \times 10^8$	$9.83 \times 10^8$	$9.74 \times 10^8$	$9.89 \times 10^8$

\*Total Viable Bacterial Counts(TVBC)

In case of Rui (*Labeo rohita*) fishes, Ponds samples ranged from  $2.75 \times 10^5$  to  $3.90 \times 10^5$  cfu/g in first day and reached up to  $9.62 \times 10^7$  cfu/g in 30<sup>th</sup> days. In wholesale market samples Total viable bacterial counts ranged from  $4.60 \times 10^5$  to  $5.23 \times 10^5$  in first day and reached up to  $9.50 \times 10^8$  cfu/g at the 30<sup>th</sup> day. Local Retail Market always shows higher counts than other two environmental conditions. It started from  $1.89 \times 10^6$  to  $5.10 \times 10^6$  cfu/g in first day and reached up to  $9.89 \times 10^8$  cfu/g in local retail market samples. So, it shows higher range of Total Bacterial Counts in local retail market samples

Table 2: Bacteriological quality assessments of Catla (*Catla catla*) fish samples collected from ponds, wholesale market, local retail markets for 5 days interval up to 30days.

Days	Parameters	Comilla pond-1 (cfu/g)	Comilla pond-2 (cfu/g)	Comilla pond-3 (cfu/g)	Whole sale Market-1 (cfu/g)	Wholesale Market-2 (cfu/g)	Wholesale Market-3 (cfu/g)	Local Retail Market-1 (cfu/g)	Local Retail Market-2 (cfu/g)	Local Retail Market-3 (cfu/g)
0	TVBC	$3.50 \times 10^5$	$3.30 \times 10^5$	$3.82 \times 10^5$	$4.62 \times 10^5$	$4.36 \times 10^5$	$4.52 \times 10^5$	$4.03 \times 10^6$	$3.50 \times 10^6$	$3.60 \times 10^6$
5	TVBC	$4.69 \times 10^5$	$5.36 \times 10^5$	$5.12 \times 10^5$	$5.50 \times 10^5$	$6.02 \times 10^5$	$7.34 \times 10^5$	$5.15 \times 10^6$	$4.59 \times 10^6$	$6.23 \times 10^6$

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10	TVBC	$5.36 \times 10^5$	$6.22 \times 10^5$	$5.29 \times 10^5$	$6.44 \times 10^5$	$6.44 \times 10^5$	$7.937 \times 10^5$	$7.56 \times 10^6$	$6.33 \times 10^6$	$7.33 \times 10^6$
15	TVBC	$6.12 \times 10^5$	$7.32 \times 10^5$	$8.93 \times 10^5$	$8.21 \times 10^5$	$7.64 \times 10^5$	$8.36 \times 10^5$	$9.61 \times 10^6$	$7.98 \times 10^6$	$8.14 \times 10^6$
20	TVBC	$6.96 \times 10^5$	$7.42 \times 10^5$	$9.11 \times 10^5$	$8.29 \times 10^7$	$8.33 \times 10^7$	$8.93 \times 10^7$	$8.36 \times 10^7$	$8.23 \times 10^7$	$8.87 \times 10^7$
25	TVBC	$7.92 \times 10^7$	$9.32 \times 10^7$	$9.26 \times 10^7$	$9.22 \times 10^8$	$9.23 \times 10^8$	$9.16 \times 10^8$	$9.31 \times 10^8$	$9.55 \times 10^8$	$9.32 \times 10^8$
30	TVBC	$9.36 \times 10^7$	$9.98 \times 10^7$	$9.82 \times 10^7$	$9.29 \times 10^8$	$9.37 \times 10^8$	$9.25 \times 10^8$	$9.82 \times 10^8$	$9.93 \times 10^8$	$9.67 \times 10^8$

In first day, Total Viable Bacterial Count of Catla (*Catla catla*) ranged from  $3.30 \times 10^5$  to  $3.82 \times 10^5$  cfu/g for ponds samples. In 30<sup>th</sup> days it ranged upto  $9.98 \times 10^7$  cfu/g in Comilla ponds sample-2 fishes. In case of Wholesale market sample of Catla (*Catla catla*) counts ranged from  $4.36 \times 10^5$  to  $4.62 \times 10^5$  cfu/g and ranged upto  $9.79 \times 10^8$  cfu/g in wholesale market-2 samples of Catla (*Catla catla*). These Wholesale market samples always shows relatively higher microbial load than Ponds samples. For Local Retail Market samples lowest counts found is  $3.50 \times 10^6$  cfu/g Local Retail Market-2 Sample and highest  $4.03 \times 10^6$  cfu/g in Local Retail Market-1. It shows up to  $9.59 \times 10^8$  cfu/g counts in  $9.63 \times 10^8$  cfu/g in Local Retail Market-2 sample.

Table 3: Bacteriological quality assessments of Tilapia (*Oreochromis mossambicus*) fish samples collected from ponds, wholesale market, local retail markets for 5 days interval up to 30 days.

Days	parameters	Comilla pond-1 (cfu/g)	Comilla pond-2 (cfu/g)	Comilla pond-3 (cfu/g)	Whole sale market-1 (cfu/g)	Wholesale market-2 (cfu/g)	Wholesale market-3 (cfu/g)	Local Retail market-1 (cfu/g)	Local Retail market-2 (cfu/g)	Local Retail market-3 (cfu/g)
0	TVBC	$3.21 \times 10^5$	$3.75 \times 10^5$	$3.92 \times 10^5$	$5.75 \times 10^5$	$5.72 \times 10^5$	$5.75 \times 10^5$	$5.60 \times 10^6$	$4.72 \times 10^6$	$6.29 \times 10^6$
5	TVBC	$3.63 \times 10^5$	$4.51 \times 10^5$	$4.98 \times 10^5$	$6.55 \times 10^5$	$6.02 \times 10^5$	$6.34 \times 10^5$	$6.45 \times 10^6$	$4.96 \times 10^6$	$6.63 \times 10^6$
10	TVBC	$4.06 \times 10^5$	$5.82 \times 10^5$	$5.19 \times 10^5$	$6.94 \times 10^5$	$6.44 \times 10^5$	$7.37 \times 10^5$	$7.56 \times 10^6$	$5.23 \times 10^6$	$7.11 \times 10^6$
15	TVBC	$4.22 \times 10^5$	$6.32 \times 10^5$	$6.23 \times 10^5$	$7.31 \times 10^5$	$7.25 \times 10^5$	$7.23 \times 10^5$	$7.88 \times 10^6$	$6.18 \times 10^6$	$8.04 \times 10^6$
20	TVBC	$5.63 \times 10^5$	$6.42 \times 10^5$	$7.01 \times 10^5$	$8.19 \times 10^7$	$7.56 \times 10^7$	$7.53 \times 10^7$	$8.36 \times 10^7$	$7.89 \times 10^7$	$8.67 \times 10^7$
25	TVBC	$6.12 \times 10^7$	$7.32 \times 10^7$	$8.26 \times 10^7$	$8.22 \times 10^8$	$8.36 \times 10^8$	$8.46 \times 10^8$	$8.69 \times 10^8$	$8.05 \times 10^8$	$9.02 \times 10^8$
30	TVBC	$7.36 \times 10^7$	$8.03 \times 10^7$	$9.22 \times 10^7$	$8.92 \times 10^8$	$8.79 \times 10^8$	$9.00 \times 10^8$	$9.62 \times 10^8$	$9.83 \times 10^8$	$9.96 \times 10^8$

Among the 3 ponds samples of Tilapia (*Oreochromis mossambicus*) of different ponds of Comilla, TVBC ranging from  $3.21 \times 10^5$  to  $3.92 \times 10^5$  cfu/g which is within the limit  $1 \times 10^6$  cfu/g set by International Commission on Microbiology Specifications for Foods but it ranged up to  $9.22 \times 10^7$  cfu/g to Comilla pond-3 sample. In Wholesale Market samples, Total Viable Bacterial counts ranged from  $5.72 \times 10^5$  to  $5.75 \times 10^5$  cfu/g and the highest bacterial presence was found  $9.79 \times 10^8$  cfu/g in Wholesale Market-2 Tilapia samples in 30<sup>th</sup> day. In first day, Total Viable Bacterial counts ranged from  $4.72 \times 10^6$  to  $6.29 \times 10^6$  cfu/g and highest  $9.36 \times 10^8$  cfu/g found in Local Retail market-3 tilapia samples in 30<sup>th</sup> days. Total bacterial counts gradually increase in all samples day by days.

Table 4: Bacteriological quality assessments of Pangas (*Pangasianodon hypophthalmus*) fish samples collected from ponds, wholesale market, and local retail markets for 5 days interval up to 30<sup>th</sup> day.

Days	parameters	Comilla pond-1 (cfu/g)	Comilla pond-2 (cfu/g)	Comilla pond-3 (cfu/g)	Whole sale market-1 (cfu/g)	Wholesale market-2 (cfu/g)	Wholesale market-3 (cfu/g)	Local Retail market-1 (cfu/g)	Local Retail market-2 (cfu/g)	Local Retail market-3 (cfu/g)
0	TVBC	$3.14 \times 10^5$	$2.9 \times 10^5$	$3.55 \times 10^5$	$4.60 \times 10^5$	$6.50 \times 10^5$	$7.13 \times 10^5$	$2.39 \times 10^6$	$3.66 \times 10^6$	$5.60 \times 10^6$
5	TVBC	$3.66 \times 10^5$	$3.11 \times 10^5$	$4.98 \times 10^5$	$4.55 \times 10^5$	$6.70 \times 10^5$	$7.34 \times 10^5$	$2.85 \times 10^6$	$4.56 \times 10^6$	$6.13 \times 10^6$
10	TVBC	$4.26 \times 10^5$	$4.32 \times 10^5$	$5.29 \times 10^5$	$5.14 \times 10^5$	$7.14 \times 10^5$	$7.37 \times 10^5$	$3.46 \times 10^6$	$5.03 \times 10^6$	$6.29 \times 10^6$

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15	TVBC	4.52x10 <sup>5</sup>	5.12x10 <sup>5</sup>	6.73x10 <sup>5</sup>	5.21x10 <sup>5</sup>	7.89x10 <sup>5</sup>	8.33x10 <sup>5</sup>	4.43x10 <sup>6</sup>	6.58x10 <sup>6</sup>	8.24x10 <sup>6</sup>
20	TVBC	5.33x10 <sup>5</sup>	6.42x10 <sup>5</sup>	7.18x10 <sup>5</sup>	6.29x10 <sup>7</sup>	8.05x10 <sup>7</sup>	8.24x10 <sup>7</sup>	5.26x10 <sup>7</sup>	7.03x10 <sup>7</sup>	8.88x10 <sup>7</sup>
25	TVBC	6.12x10 <sup>7</sup>	7.32x10 <sup>7</sup>	8.86x10 <sup>7</sup>	7.22x10 <sup>7</sup>	9.34x10 <sup>7</sup>	8.36x10 <sup>7</sup>	6.11x10 <sup>8</sup>	8.15x10 <sup>8</sup>	9.12x10 <sup>8</sup>
30	TVBC	7.36x10 <sup>7</sup>	8.23x10 <sup>7</sup>	9.62x10 <sup>7</sup>	9.69x10 <sup>7</sup>	9.98x10 <sup>7</sup>	1.59x10 <sup>8</sup>	6.19x10 <sup>8</sup>	9.63x10 <sup>8</sup>	9.68x10 <sup>8</sup>

Pangas (*Pangasianodon hypophthalmus*) samples of Comilla ponds shows relatively lowest Bacterial compared to other to environmental variations. Total Bacterial Counts started from 2.9x10<sup>5</sup> cfu/g to 3.55x10<sup>5</sup> cfu/g in first days and shows highest values 8.23x10<sup>7</sup> cfu/g in Comilla pond-2 in all ponds samples of Pangas (*Pangasianodon hypophthalmus*) in 30<sup>th</sup> days. For Wholesale Market samples of Pangas bacterial counts ranged from 4.60x10<sup>5</sup> to 7.13x10<sup>5</sup> cfu/g in first days and highest bacterial counts found in 9.91x10<sup>8</sup> cfu/g in Wholesale market-2 Pangas samples at 30<sup>th</sup> day. In Local Market samples of Pangas total bacterial counts ranged from 2.39x10<sup>6</sup> to 5.60x10<sup>6</sup> cfu/g in first days and highest bacterial counts found in 9.68x10<sup>8</sup> cfu/g in Local Retail Market-3 Pangas samples at 30<sup>th</sup> day.

Table 5: Bacteriological quality assessments of Mrigal (*Cirrhinus cirrhosus*) fish samples collected from ponds, wholesale market, local retail markets for 5 days interval up to 30<sup>th</sup> day.

Day	parameters	Comilla pond-1 (cfu/g)	Comilla pond-2 (cfu/g)	Comilla pond-3 (cfu/g)	Whole sale market-1 (cfu/g)	Wholesale market-2 (cfu/g)	Wholesale market-3 (cfu/g)	Local Retail market-1 (cfu/g)	Local Retail market-2 (cfu/g)	Local Retail market-3 (cfu/g)
0	TVBC	3.61x10 <sup>5</sup>	3.60x10 <sup>5</sup>	4.22x10 <sup>5</sup>	5.32x10 <sup>5</sup>	6.26x10 <sup>5</sup>	5.22x10 <sup>5</sup>	3.06x10 <sup>6</sup>	3.92x10 <sup>6</sup>	4.71x10 <sup>6</sup>
5	TVBC	3.89x10 <sup>5</sup>	4.36x10 <sup>5</sup>	5.23x10 <sup>5</sup>	5.63x10 <sup>5</sup>	6.50x10 <sup>5</sup>	5.63x10 <sup>5</sup>	3.45x10 <sup>6</sup>	4.06x10 <sup>6</sup>	5.23x10 <sup>6</sup>
10	TVBC	4.23x10 <sup>5</sup>	5.36x10 <sup>5</sup>	6.19x10 <sup>5</sup>	6.36x10 <sup>5</sup>	7.22x10 <sup>5</sup>	7.37x10 <sup>5</sup>	3.56x10 <sup>6</sup>	4.33x10 <sup>6</sup>	6.29x10 <sup>6</sup>
15	TVBC	4.32x10 <sup>5</sup>	5.92x10 <sup>5</sup>	6.93x10 <sup>5</sup>	7.63x10 <sup>5</sup>	7.69x10 <sup>5</sup>	8.13x10 <sup>5</sup>	4.88x10 <sup>6</sup>	5.98x10 <sup>6</sup>	7.84x10 <sup>6</sup>
20	TVBC	5.33x10 <sup>5</sup>	6.42x10 <sup>5</sup>	7.11x10 <sup>5</sup>	8.19x10 <sup>7</sup>	8.10x10 <sup>7</sup>	8.23x10 <sup>7</sup>	8.66x10 <sup>7</sup>	6.23x10 <sup>7</sup>	8.87x10 <sup>7</sup>
25	TVBC	6.11x10 <sup>7</sup>	7.32x10 <sup>7</sup>	8.06x10 <sup>7</sup>	8.22x10 <sup>7</sup>	8.36x10 <sup>7</sup>	9.06x10 <sup>7</sup>	6.67x10 <sup>8</sup>	7.57x10 <sup>8</sup>	9.09x10 <sup>8</sup>
30	TVBC	7.36x10 <sup>7</sup>	8.23x10 <sup>7</sup>	9.23x10 <sup>7</sup>	9.39x10 <sup>7</sup>	9.29x10 <sup>7</sup>	9.59x10 <sup>7</sup>	7.72x10 <sup>8</sup>	8.63x10 <sup>8</sup>	9.69x10 <sup>8</sup>

We found 3.60x10<sup>5</sup> to 4.22x10<sup>5</sup> cfu/g Total Bacterial Counts in Comilla pond samples of Mrigal (*Cirrhinus cirrhosus*) in first day and ranged up to 9.23x10<sup>7</sup> cfu/g in 30<sup>th</sup> days. In wholesale market samples shows relatively microbial load and ranged upto 9.59x10<sup>8</sup> cfu/g In Local market samples of Mrigal (*Cirrhinus cirrhosis*) Total bacterial loads ranged upto 9.69x10<sup>8</sup> cfu/g Local Retail Market-2 sample.

Table 6: Bacteriological quality assessments of Carfu (*Cyprinus carpio*) fish samples collected from ponds, wholesale market, local retail markets for 5 days interval up to 30<sup>th</sup> day.

Days	parameters	Comilla pond-1 (cfu/g)	Comilla pond-2 (cfu/g)	Comilla pond-3 (cfu/g)	Whole sale market-1 (cfu/g)	Wholesale market-2 (cfu/g)	Wholesale market-3 (cfu/g)	Local Retail market-1 (cfu/g)	Local Retail market-2 (cfu/g)	Local Retail market-3 (cfu/g)
0	TVBC	3.31x10 <sup>5</sup>	3.91x10 <sup>5</sup>	4.62x10 <sup>5</sup>	6.10x10 <sup>5</sup>	4.32x10 <sup>5</sup>	7.45x10 <sup>5</sup>	3.52x10 <sup>6</sup>	4.12x10 <sup>6</sup>	5.92x10 <sup>6</sup>
5	TVBC	4.06x10 <sup>5</sup>	4.21x10 <sup>5</sup>	5.08x10 <sup>5</sup>	6.55x10 <sup>5</sup>	5.12x10 <sup>5</sup>	7.84x10 <sup>5</sup>	3.75x10 <sup>6</sup>	4.56x10 <sup>6</sup>	6.22x10 <sup>6</sup>
10	TVBC	4.56x10 <sup>5</sup>	4.62x10 <sup>5</sup>	6.11x10 <sup>5</sup>	6.74x10 <sup>5</sup>	5.49x10 <sup>5</sup>	7.89x10 <sup>5</sup>	3.96x10 <sup>6</sup>	5.33x10 <sup>6</sup>	6.49x10 <sup>6</sup>

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15	TVBC	$5.12 \times 10^5$	$5.32 \times 10^5$	$6.93 \times 10^5$	$7.91 \times 10^5$	$6.99 \times 10^5$	$8.13 \times 10^5$	$4.11 \times 10^6$	$5.98 \times 10^6$	$7.34 \times 10^6$
20	TVBC	$5.33 \times 10^5$	$7.52 \times 10^5$	$7.17 \times 10^5$	$8.79 \times 10^7$	$7.06 \times 10^7$	$8.23 \times 10^7$	$9.36 \times 10^7$	$9.13 \times 10^7$	$9.87 \times 10^7$
25	TVBC	$6.67 \times 10^7$	$8.12 \times 10^7$	$8.46 \times 10^7$	$9.12 \times 10^7$	$8.36 \times 10^7$	$9.34 \times 10^7$	$6.49 \times 10^8$	$7.55 \times 10^8$	$8.32 \times 10^8$
30	TVBC	$7.36 \times 10^7$	$9.43 \times 10^7$	$9.22 \times 10^7$	$9.89 \times 10^7$	$9.29 \times 10^7$	$9.50 \times 10^7$	$7.62 \times 10^8$	$8.63 \times 10^8$	$8.89 \times 10^8$

From this study, it shows that among the 3 samples of Carfu (*Cyprinus carpio*) TVBC started from  $3.31 \times 10^5$  to  $4.62 \times 10^5$  in ponds sample in first day they ranged upto  $9.43 \times 10^7$  cfu/g in 30<sup>th</sup> day. In wholesale market samples and Local Retail Market samples TVBC ranged up to  $9.89 \times 10^8$  cfu/g and TVBC  $8.89 \times 10^8$  cfu/g respectively.

### B. Chemical Analysis

FFA (Free Fatty Acid), POV (Per-oxide Value), IV (Iodine Value) is used to estimate the breakdown components and hydrolytic and oxidative degradation of the fatty components of fish during storage [10]. Average FFA is highest in Local retail market samples. We can see that FFA increased with the environmental conditions and day by Day. POV is also increased with environmental condition and day by day in all selected samples. But Iodine value is decreased with environmental condition and day by day.

Table 7: Changes in chemical parameters of fats of Rui (*Labeo rohita*), Catla (*Catla catla*), Tilapia (*Oreochromis mossambicus*) upto 30 days

Days	Parameters	Rui			Catla			Tilapia		
		CP AV	WM AV	LM AV	CP AV	WM AV	LM AV	CP AV	WM AV	LM AV
0	FFA (% as oleic)	0.46	0.47	0.49	0.48	0.52	0.63	0.46	0.47	0.51
	POV (m. eq. Kg <sup>-1</sup> )	7.89	8.10	8.12	8.30	8.52	8.66	7.93	8.13	8.14
	IV	84.86	84.50	84.22	84.49	83.21	78.23	84.88	83.88	82.74
5	FFA (% as oleic)	0.55	0.59	0.61	0.65	0.72	0.75	0.55	0.65	0.69
	POV (m. eq. Kg <sup>-1</sup> )	14.58	18.78	18.78	18.78	18.78	18.78	14.58	18.78	18.78
	IV	80.41	78.23	75.12	80.96	76.32	74.25	80.41	78.63	76.32
10	FFA (% as oleic)	0.59	0.88	1.00	0.88	0.95	1.12	0.59	0.78	0.88
	POV (m. eq. Kg <sup>-1</sup> )	14.31	16.40	16.40	16.40	16.40	16.40	14.31	16.40	16.40
	IV	80.13	76.22	73.26	79.43	75.44	73.12	80.13	79.85	72.32
15	FFA (% as oleic)	0.66	0.95	1.23	0.95	1.32	1.52	0.66	0.95	1.00
	POV (m. eq. Kg <sup>-1</sup> )	15.96	19.74	19.74	19.74	19.74	19.74	15.96	19.74	19.74
	IV	78.92	74.23	72.65	75.35	73.11	72.56	78.92	75.35	72.65
20	FFA (% as oleic)	0.75	1.33	1.45	1.33	1.48	1.53	0.75	1.33	1.52
	POV (m. eq. Kg <sup>-1</sup> )	17.50	22.16	22.16	22.16	22.16	22.16	17.50	22.16	22.16
	IV	74.32	71.88	70.33	71.87	70.96	69.22	74.32	71.87	70.22
25	FFA (% as oleic)	0.82	1.40	1.54	1.40	1.45	1.58	0.82	1.40	1.56

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	POV (m. eq. Kg <sup>-1</sup> ) <sub>1</sub>	20.21	16.49	16.49	16.49	16.49	16.49	20.21	16.49	16.49
	IV	71.52	70.11	69.36	66.11	63.23	62.89	71.52	66.11	65.32
30	FFA (% as oleic)	1.02	1.45	1.57	1.45	1.49	1.52	1.02	1.45	1.53
	POV (m. eq. Kg <sup>-1</sup> ) <sub>1</sub>	12.27	15.84	15.84	15.84	15.84	15.84	12.27	15.84	15.84
	IV	69.69	69.04	64.70	64.89	62.29	61.08	69.58	65.05	64.88

From the results we can see that FFA Average of Rui, Catla, tilapia started at 0.46 (% as oleic) at the first days and ranged upto 1.57, 1.52, 1.53 (% as oleic) respectively in 30<sup>th</sup> day. POV Average ranged from 7.89 to 15.84 (m. eq. Kg<sup>-1</sup>) in Rui, 8.30 to 15.82 (m. eq. Kg<sup>-1</sup>) in Catla and 7.93 to 5.84 (m. eq. Kg<sup>-1</sup>) in Tilapia. Iodine value Average ranged from 84.86 to 63.88 in all three samples.

Table 8: Changes in chemical parameters of fats of Pangas (*Pangasianodon hypophthalmus*), Mrigal (*Cirrhinus cirrhosus*) and Carfu (*Cyprinus carpio*) upto 30 days

Days	Parameters	Pangas			Mrigal			Carfu		
		CP AV	WM AV	LM AV	CP AV	WM AV	LM AV	CP AV	WM AV	LM AV
0	FFA (% as oleic)	0.48	0.56	0.71	0.46	0.47	0.50	0.48	0.49	0.56
	POV (m. eq. Kg <sup>-1</sup> ) <sub>1</sub>	8.30	8.54	8.66	7.73	8.21	8.24	8.30	8.62	8.66
	IV	84.49	83.17	80.23	84.88	82.36	80.63	84.49	82.63	80.85
5	FFA (% as oleic)	0.65	0.72	0.75	0.55	0.58	0.62	0.65	0.68	0.71
	POV (m. eq. Kg <sup>-1</sup> ) <sub>1</sub>	18.78	18.78	18.78	8.30	8.93	9.10	18.78	18.78	18.78
	IV	80.96	78.32	76.32	80.41	78.36	75.65	80.96	79.65	74.36
10	FFA (% as oleic)	0.88	1.02	1.22	0.59	0.75	0.87	0.88	1.02	1.22
	POV (m. eq. Kg <sup>-1</sup> ) <sub>1</sub>	16.40	16.40	16.40	9.56	10.54	11.36	16.40	16.40	16.40
	IV	79.43	76.35	72.32	80.13	79.43	72.36	79.43	78.22	76.35
15	FFA (% as oleic)	0.95	0.98	1.22	0.66	0.93	1.00	0.95	0.98	1.23
	POV (m. eq. Kg <sup>-1</sup> ) <sub>1</sub>	19.74	19.74	19.74	12.32	15.36	16.56	19.74	19.74	19.74
	IV	75.35	73.23	71.65	78.92	75.35	74.32	75.35	73.26	72.39
20	FFA (% as oleic)	1.33	1.42	1.58	0.74	1.33	1.34	1.33	1.39	1.42
	POV (m. eq. Kg <sup>-1</sup> ) <sub>1</sub>	22.16	22.16	22.16	13.23	16.42	17.65	22.16	22.16	22.16
	IV	71.87	68.52	67.32	74.32	71.87	69.33	71.87	70.22	69.32
25	FFA (% as oleic)	1.40	1.63	1.72	0.83	1.45	1.52	1.40	1.52	1.63
	POV (m. eq. Kg <sup>-1</sup> ) <sub>1</sub>	16.49	16.49	16.49	14.58	17.31	18.62	16.49	16.49	16.49
	IV	66.11	63.21	62.11	71.52	69.32	66.11	66.11	65.32	64.65
30	FFA (% as oleic)	1.45	1.63	1.72	1.02	1.54	1.63	1.45	1.77	1.85
	POV (m. eq. Kg <sup>-1</sup> ) <sub>1</sub>	15.84	15.84	15.84	15.36	18.96	19.68	15.84	15.84	15.84
	IV	65.16	62.11	61.22	69.89	66.36	64.36	65.06	63.32	62.19

Form the Table 8 we can see that that FFA Average of Pangas, Mrigal, Carfu started at 0.48, 0.46, 0.48 (% as oleic) at the first days and ranged upto 1.63, 1.63, 1.77, 1.53 (% as oleic) respectively in 30<sup>th</sup> day. POV ranged from 8.30, 8.93 to 15.84 (m. eq. Kg<sup>-1</sup>) in

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Pangas, 8.30 to 15.82(m. eq. Kg<sup>-1</sup>) in Mrigal and 7.93 to 5.84 (m. eq. Kg<sup>-1</sup>) in Carfu within 30 days. Within 30 days Iodine value ranged from 84.88 to 62.19 in Pangas, Mrigal, Carfu.

### IV. CONCLUSION

The results revealed that microbiological and chemical quality of the fishes influenced by initial quality of the fishes. Initial quality of the fish to begin with should be as good as possible for its use as a raw material for frozen storage. From the results, it can be concluded that fishes from cultured ponds are always good in quality in respect to the microbiological, chemical test even after 30 days storage in freeze.

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