Seasonal variations in biochemical and microbiological quality of three important dried fishes from Tripura market

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Abstract
In the present study, seasonal variations in physico-chemical and microbiological quality of three dried fishes namely Chandana (Hilsa toli), Ghol fish (Johnius caruta) and Bombay duck (Harpadon nehereus) were assessed in monsoon, winter and summer seasons. The physicochemical and microbiological quality of the experimental fishes were good in winter and summer season. However, a considerable quality deterioration was seen in the monsoon season in all the three experimental fishes. Among the experimental species, H. toli had considerable variation in biochemical quality than that of H. nehereus and J. caruta. The higher relative humidity coupled with temperature in monsoon season were the critical factors in order to deteriorate physico-chemical and microbiological quality of dried fishes as revealed by correlation coefficients between humidity and quality parameters. The absence of the pathogenic strains of faecal coliform and salmonella in experimental fishes indicated that fishes were devoid of external contamination and found fit for human consumption.

Keywords: dried fish, hilsa toil, harpadon nehereus, johnius caruta, quality, north-east region

1. Introduction
Dried fish is very cheap source of concentrated protein and a very popular food item in north eastern region of India (NER). People in NER are very fond of dried fishes and eat them on almost daily basis. Although, it is a very well-known fact that fish is highly perishable commodity even in dried condition. Therefore, quality of the dried fish always remains doubtful. However, there are many methods of fish preservation in vogue such as icing, freezing, canning, salting, smoking and drying to preserve for future use.

Drying is one of world’s oldest known food preservation method and still being practiced in many parts of the world. It works by removing water from food, which inhibits the growth of microorganisms. About 8 million tons of fish (25-30%) of the world catch for human consumption are dried, salted, smoked or treated by some combination of these processes each year. Smoking, drying and curing of fish either as a means of prolonging shelf life, or to produce desired flavours and texture has been practiced by many societies for centuries. Though, most of fish produced in India, either marine or freshwater, consumed as fresh as possible but an appreciable amount of the fish is dried using various traditional and modern drying methods especially in northeast India. In India about 17% of the total catch is being used for the production of dry fishes Shakila et al. (2003) [31]. The consumption of dried fishes is about 32 % of the total marine landings in India. As per the recent report published by Marine Product Export Development Authority (MPEDA), more than Rs.725 crore worth dried items exported to various part of the world MPEDA (2017) [27]. Compared to other processing methods, drying is quite simple and dried fish has a considerable storage life that too at room temperature.

In north-eastern region of India, especially in Tripura state, fish is the major source of animal protein for the people and 95% people consume fish. Its per capita fish consumption is very high (i.e.13 kg) compared to national average (9.2 kg) and compelled to solve the demand by importing fresh as well as dried fish from other states and neighbouring country Bangladesh. The aboriginal people of the area consider various fish species as a major source of protein food Hazarika et al. (2016) [17]. The dried fish are popular choice of most of the in habitants especially tribal population of the state and contribute a major source of fish protein. Since the
ethnic populace of the state live in hilly areas, therefore, dry fish becomes advantageous for them as these could be stored for a long period of time and consumed as and when desired. In India, particularly in northeast region, drying remains an important fish preservation method since time immemorial. During monsoon months, different species of fresh water fish are caught from the rivers and ‘beels’ (a natural bounded water body) spread across the NER. A bulk amount of such catch is traditionally dried by different methods. Several researchers reported about the nutritional quality of dried fish such as dried tilapia, dried Mystus vittatus, Channa punctatus, Chanda nama, Corica soborna and Trichirius haumela, sun-dried Labeo rohita, Labeo gonius, snake headed fish (Channa striatus) and eurasian catfish (Wallago attu). Mansur et al. (2013) [26], Chinese pomfret (Stromateus chinchisnis), Bombay duck (Harpadon nehereus) and ribbon fishes (Trichirius haumela) Pravakar et al. (2013) [30] and dried fishes of northeast region of India Hazarika et al. (2016) [17] and Bangladesh. Although, there are reports of quality evaluation of particular variety of dried fish of the retail market of different states, but a systematic study on the changes of biochemical as well as microbial quality of dried fish of the retail market with the season is lacking. Many varieties of dried fish of both freshwater and marine origin are sold in the retail dry fish markets of the state, but of which most popular ones are Bombay duck (Harpadon nehereus), Ghel Fish (Johnius caruta) and Chandana (Hilsa toli). These are available almost throughout the year. Therefore, seasonal assessment of proximate composition, biochemical and microbiological quality of these three most demanded dried fish has been focused in this study.

2. Materials and Methods
2.1 Materials
The salted-dried Chandana (H. toli), Ghel fish (J. caruta) and Bombay duck (H. nehereus) were used in the study. The dried fish samples were collected aseptically in sterile pouches from Gol Bazar (Local market), Agartala, India in first week of every month and brought to the laboratory for biochemical and microbiological analysis. The sampling was performed for 12 months. The fishes were purchased from randomly selected 3-4 fish shops to ensure the homogeneity of the samples. The average size and weight of H. toli, J. caruta and H. nehereus were 13.3 ± 0.3cm, 26.6 ± 1.6g, 9.0 ± 0.5cm, 19.26 ± 0.99g and 12.79 ± 0.6cm, 8.57± 0.68g respectively.

2.2 Methods
2.2.1 Proximate analysis of dried fish
The proximate composition analysis (moisture, crude protein, fat and ash content) of experimental dried fish samples was carried out using standard methods AOAC (2010) [2].

2.2.2 Estimation of pH
A 5 g fish sample was homogenized in 45 ml of CO2 free water in pestle and mortar. The pH of slurry was determined by a digital pH meter (Sartorius PB 20). The pH meter was calibrated using pH 4.0 and 7.0 buffer tablets before every measurement.

2.2.3 Estimation of Non-Protein Nitrogen
Non protein nitrogen (NPN) content was determined by the method as described by Velanker and Govindan (1958) [35]. About 10 g of sample was taken and macerated with 10 ml of 15 % TCA for 3 min. using pestle and mortar. The slurry was allowed to settle at refrigerated temperature for 30 min. The slurry was filtered and made up to 50 ml with distilled water and 20 ml aliquot was taken for nitrogen estimation by Kjeldahl method. The NPN content was expressed as mg N/ 100 g meat.

2.2.4 Estimation of Total Volatile Basic Nitrogen
Total Volatile Base Nitrogen (TVBN) content of dry fish samples was determined by Conway micro diffusion method Beatty and Gibson (1937) [5]. 10 g of the dry fish sample was macerated with 20 % tri-chloro acetic acid (TCA) solution using pestle and mortar. The slurry was filtered with coarse filter paper and made up to 100 ml with distilled water. 2 ml of boric acid containing mixed indicator (0.066% methyl red and 0.066 % bromo cresol green solution in alcohol in ratio of 1:1) was added into the inner chamber and 1 ml of sample into outer chamber of Conway micro diffusion unit followed by addition of potassium carbonate in the same chamber. Grease was applied on the covering glass of unit to make it air tight. The solution was incubated at 37 °C for 90 min. After incubation, inner chamber content was titrated against 0.02N sulphuric acid. A blank was run using 2% TCA solution instead of sample. TVBN was calculated and expressed in mg %.

2.2.5 Estimation of Trimethylamine Nitrogen
Tri-methyl amine (TMA) content of dry fish samples was determined by Conway micro diffusion method Beatty and Gibson (1937) [5]. 10 g of the dry fish sample was macerated with 20 % tri-chloro acetic acid (TCA) solution using pestle and mortar. The slurry was filtered with coarse filter paper and was made up to 100 ml with distilled water. 2 ml of boric acid containing mixed indicator (0.066% methyl red and 0.066 % bromo cresol green solution in alcohol in ratio of 1:1) into the inner chamber and 1 ml of sample into outer chamber of Conway micro-diffusion unit follow by addition of potassium carbonate and formaldehyde in the same chamber. The grease was applied on covering glass of unit to make air tight. Solution was incubated at 37 °C for 90 min. After incubation, inner chamber content was titrated against 0.02N sulphuric acid. A blank was run using 2% TCA solution instead of sample. TMA content was calculated and expressed in mg %.

2.2.6 Estimation of Peroxide Value
The Peroxide Value content was determined by the method as described by Jacob (1958) [23]. 10 g dry fish was macerated with 15 g anhydrous sodium sulphate transferred to an iodine flask. After that, 80-100ml chloroform was added followed by shaking vigorously for 2-3 min and kept in dark overnight. The slurry was filtered using Whatman No. 1 filter paper. Exactly 10ml of chloroform extract was taken in a dried and weighed Petri dish which was placed over a hot plate to evaporate chloroform and weight of the oil was determined from the difference of weight. 5-10 ml of chloroform extract was taken in a 250ml iodine flask and 25ml of solvent (2 parts by volume of glacial acetic acid and 1 part by volume of chloroform) was added to this followed by 1g potassium iodide salt. The content was shaken well for one minute and allowed to stand in dark for 30 min. Then about 35ml of distilled water was added by washing the stopper and sides of the flask. The content in the flask was titrated against 0.01N sodium thiosulphate solution using starch as indicator with vigorous shaking till complete disappearance of blue colour.
A blank was done simultaneously with solvent only.

2.2.7 Estimation of Free Fatty Acid Value
The Free fatty acid was determined by the method as described by AOAC (1995) [1]. 10 ml of chloroform extract was taken in a clean and dry conical flask. 25 ml of hot neutral alcohol was added followed by few drops of phenolphthalein indicator. The content was titrated by 0.1 N KOH with vigorous shaking to a faint pink colour of the same intensity as that of neutral alcohol before addition of the sample. The colour must persist for at least 30 sec.

2.2.8 Estimation of Thiobarbituric acid reactive substances (TBARS) content
TBARS substances content was determined by the method as described by Buege and Aust (1978) [8]. 1g of sample was taken and homogenized with 10 ml of TCA-TBA- HCl reagent (15 g of TCA and 0.375 g of TBA was added to 100 ml volumetric flask and volume was made up to 100 ml with 0.25 HCl and heated for dissolution of TBA) in a plastic centrifugal tube. Then the mixture was heated in boiling water for 15 minutes followed by cooling in running tap water. After cooling it was centrifuged at 5000 rpm for 10 minutes and precipitate was removed and absorbance was measured at 532 nm wave length. Blank was also run simultaneously. TBARS value was calculated using molar extinction coefficient value of 1.56 nM^-1 cm^-1.

3. Microbiological analysis of dried fish
3.1 Total plate count
The total plate count was determined by the method as described by APHA (1995) [9]. For enumerating total bacteria or Total plate count (TPC), 10 g each samples of dried fish were macerated with 90 ml of sterile physiological saline (0.85% NaCl). Tenfold serial dilutions of the samples were made and 0.1ml from each dilution was placed on duplicate plates of nutrient agar following spread plate technique. The plates were incubated in aerobic condition for 24 hrs. Colony counted and expressed in log _cfu_ (colony forming units) per gram of samples. The TPC was calculated using following formula

\[
\text{Count per gram} = \frac{\text{Number of colonies counted}}{\text{X reciprocal of dilution from which the colonies counted}} \times \text{X reciprocal of aliquot plated}
\]

3.2 Total Fungal Count
The total fungal count was determined by the method as described by APHA (1976) [3]. 10g of sample was introduced aseptically in a sterile stomach bag (Seward stomach BA6141CPG standard bags) and macerated for 2 min. with 90ml of sterile diluents of 0.1% peptone using a stomacher (Seward stomach 400 circulator, England). A serial decimal dilution of 10^-2 was prepared using 9ml sterile 0.1% peptone and 1ml homogenize sample and it was well mixed in cyclomixer. Then pipette 0.1ml of inoculums was spread plated on PDA plates using a sterile glass spreader. The plates were incubated at 28-30°C for 48 hrs. The plates containing 10-150 colonies were counted. The TFC was calculated using following formula

\[
\text{Count per gram} = \frac{\text{Number of colonies counted}}{\text{x reciprocal of dilution from which the colonies counted}} \times \text{x reciprocal of aliquot plate}
\]

3.3 Isolation and Identification of salmonella
_Salmonella_ was isolated by conventional method as per the protocols recommened by FDA (2001) [11]. A sample weight of 25 g was homogenized with 225 ml of lactose broth for 2 minutes. The mixture was then incubated at 37°C for 24 hrs. 1 millilitre each of pre-enriched sample was added to 10 ml each of SCB and TTB, while 0.1 ml was added to 10 ml of RV broth. The inoculated SCB and TTB were incubated at 37°C for 24 hrs, while RV broth was incubated at 43°C in water bath for 24 hrs. Subsequently a loop full of culture from each of these broths was streaked on HEA, BSA and XLD agar and incubated at 37°C for 24 hrs.

3.4 MPN procedure for the determination of total and faecal coliform counts from dried fish
The dried fish samples which used for the isolation of _Salmonella_ were also used for enumeration of total and faecal coliforms which involved presumptive and confirmed tests by FDA (2002) [12]. Dried fish samples homogenize with sterile 0.85% saline solution which was transferred to lauryl sulphate tryptone broth (LSTB) and tubes and incubated at 37°C for 48±2 hours and observed for growth and gas production. Samples from positive LSTB tubes were transferred to EC broth tubes and incubated in water bath at 37°C for 44.5 °C 24±2 hours. Calculate MPN based on proportion of EC positive tubes in 3 successive dilution using MPN table.

4. Statistical analysis
The data obtained from, microbiological and physico-chemical analysis were further analysed and interpreted using one-way ANOVA with SPSS windows 16.0 software. Karl Pearson correlation coefficient was also determined between relative humidity and microbiological, physico-chemical parameters.

5. Result and Discussion
5.1 Proximate composition analysis
5.1.1 Changes in moisture content in dried _H. toli, H. nehereus, and J. caruta_ in different seasons
The moisture content is one of the important factors that controls the quality of dried fishes. In general, the moisture content less than 15% in dried fish is considered better for longer keeping quality. However, a reduction in moisture content of fresh fish by drying to 25% will stop bacterial growth and reduce autolytic activity. In the present study, the mean moisture content of dried _H. toli, H. nehereus and J. caruta_ was 31.46 %, 22.26 % and 24.36 % respectively during the study period (2017-18). The values obtained are either near or higher to 25 % than that of reported for several dried fishes (Nath and Majumdar, 2013; Fredrick et al. 2015) [28, 13]. The moisture content of sun-dried silver pomfret (_Stromateus cincerus_) and sun-dried perch (_Lates calcarifer_) was reported very high (39.59 %) compared to our result Hossain et al. (2017) [18]. The mean relative humidity and temperature were 74.74 and 27.38 °C respectively during experimental year (Table 1).The higher moisture content in experimental fishes may be because of prevailing humid conditions and moderate temperature in Agartala throughout year.

In the present investigation, the moisture content of all three dried fishes (_H. toli, H. nehereus and J. caruta_) increased in monsoon season while decreased in winter season and remained almost static in summer season (Table 2).The mean relative humidity, in the monsoon months was higher
(84.43%) followed by winter (71.79%) and summer months (66.71%). The mean temperature in monsoon (29.40 °C) and summer (29.29 °C) months was almost similar, whereas, remained lower in winter (24.62 °C) months. Since the dried fishes are displayed in the market for sale and prevailing high humid conditions especially in monsoon season might be the reason that moisture content showed an increasing trend.

In winter season, the decrement of moisture may be because of low relative humidity and higher fat content in said season (Table 2). The inverse relationship between fat and moisture is well established. However, the moisture content of the dried fish may also depend on length of drying period, season it got caught and other treatments such as salting, smoking etc.

5.1.2 Changes in protein content Dried H. toli, H. nehereus, and J. caruta in different Seasons

The importance characteristics of dried fish is being high and concentrated proteins that contains all standard amino acids. Among the studied of three dried H. toli, H. nehereus, J. caruta mean protein contents were found to be 4.79%, 60.03%, and 56.43% respectively. Normally, the sun dried fishes contain 60-80% protein (Haque, 2004). Almost similar protein content was reported in many dried fish species in Bangladesh Mansur et al. (2013) [20].

In this study, the protein content of three dried fishes was reduced during monsoon season (Table 2). This may be due to increase in moisture content of dried fishes during monsoon season and utilization of protein by microbes for their nutrition. The protein content was higher in summer and winter seasons compared to monsoon season in all dried fishes (Table 2). Protein content was increasing during summer and winter season may be due to fish product dehydration, which might cause concentration in protein.

There was a significant (p< 0.05) negative correlation values (r = -0.97, -0.99 and -0.96 for H. toli, H. nehereus and J. caruta respectively) were observed between moisture and protein during all three seasons. This clearly indicates that in dried fish, moisture and protein had an inverse relationship. A similar findings were reported for three dried fishes (H. nehereus, J. dussumieri and L. savala) in different seasons Siddique et al. (2012) [32]. This is important to note that the mean relative humidity, in the monsoon months were higher (84.43%) followed by winter (71.79 %) and summer (66.71 %) months. In addition to the mean temperature in monsoon (29.40 °C) and summer (29.29 °C) months were almost similar and lower in winter (24.62 °C) months (Table 1).

5.1.3 Changes in lipid content dried H. toli, H. nehereus, and J. caruta in different Seasons

The means lipid content was significantly (p< 0.05) higher in H. toli (10.39 %) followed by J. caruta (7.06%) and H. Nehereus (5.84 %). Fish feed, habitat and geographical locations influences the protein and fat content of fish. In dried fishes, the moisture content is reduced noticeably, due to evaporation of water from fish muscles hence, the fat content remains slightly higher than that of fresh fish. In another study on H. nehereus, the fat content was found to be slightly higher compared to the present study. Pravakar et al. (2013) [180] revealed that lipid content of three marine dried fishes (Chinese pomfret, Bombay duck and Ribbon fish) was around 10 -12 %.

In the present study, reduction in lipid content of three experimental species during monsoon season (Table 2) could be attributed to increment in moisture content along with oxidation of poly-unsaturated fatty acids present in fish tissue to products such as peroxides, aldehydes, ketones and the free fatty acids. However, the rate of fat deterioration was very gradual. Fish oil has been found to be more liable to spoilage than other oils due to their greater number of unsaturated fatty acids. In winter season, the lipid content of three experiment species increased significantly (p< 0.05) that may be attributed to a significant (p< 0.05) negative correlation (-0.88, -0.99 and -0.85) observed between moisture and lipid. In summer again there was slight decrease in all the cases that may be related to oxidation because higher temperature observed in summer season compared to monsoon and winter seasons. A significant (p< 0.05) negative correlation values between moisture and lipid were also reported for three dried fishes (H. nehereus, J. dussumieri and L. savala) in different seasons Siddique et al. (2012) [22].

5.1.4 Changes in ash content Dried H. toli, H. nehereus, and J. caruta in different Seasons

In the present investigation, H. toli had higher ash content compared to H. nehereus and J. caruta in all three different season (Table 2). The higher ash content was estimated in three dry fishes this may be due to previous dry salting step prior to sun drying specially in H. toli and presence of sufficient pin bones (H. nehereus, J. caruta) which could not be separated during collection of flesh for analysis. The ash or mineral content of the dried fish remains slightly higher because it includes whole fish having bones, skin and scales. In case of H. toli, there was no significant (p< 0.05) change documented in monsoon and summer season, but the content slightly increased from 13.22 to 14.11 % in winter season (Table 2). Similarly, the ash content for J. caruta also had no significant (p< 0.05) change in monsoon and summer season, but the content slightly increased from 11.66 to 12.77 % in winter season (Table 2). In case of H. nehereus too, pattern was similar to that of above two fishes during different seasons. A wide range of ash content have been documented in various dried fish in earlier studies such as for dried tilapia fish (6.75-7.85%) Chinese pomfret, Bombay duck and Ribbon fish (29.19 to 11.17%) Pravakar et al. (2013) [30], Stelophorus heterolobus (nearly 9-10 %).

5.2 Biochemical quality of dried fishes

5.2.1 Changes in pH of dried H. toli, H. nehereus, and J. caruta in different Seasons

pH is an indicator of the degree of freshness or spoilage in fish. In the present study, the pH values of dry fishes H. toli, H. nehereus and J. caruta were increased from 5.83 to 6.95, 5.89 to 6.96, and 5.52 to 6.96 respectively in the monsoon season. This increment in pH may be attributed to decomposition of nitrogenous compounds during post mortem period and also the production of basic components which may induced the growth of bacteria. Fredrick et al. (2015) [13] reported that the pH values in several dried fishes (Chirocentrus dorab, Saurida tumbil, Epinephelus merra, Sillago sihama, Atule mate and Aprion virescens) that ranged from 5.10 - 6.65. It also stated that the sundried Chela fish had pH range varies from 6.6 to 7.5 at different storage period. In the present investigation, the value for H. nehereus showed an increasing (5.89 to 6.53) trend from monsoon to winter season whereas in case of J. caruta, there was no significant (p< 0.05) changes (5.52 to 5.51) were observed during three different seasons.
5.2.2 Changes in TVBN in dried H. toli, H. nehereus, and J. caruta in different Seasons

TVBN is a term that includes measurement of TMA, DMA, ammonia and other compounds associated with spoilage of fish and fish products which increases as spoilage progresses. In case of dried fish, spoilage is considered when TVBN content is 100-200mg% on dry weight basis Connell (1980) [9] similarly, the value for fresh fish remains 35-40 mgN/100 g Connell (1995) [10]. If 100-200mg% is taken as acceptable in dried fishes, the experimental dried fishes found to be fit for human consumption in all the seasons (Table 2).

In present investigation, the TVBN values of dried fishes (H. toli, H. nehereus, J. caruta) were higher in monsoon season compared to winter and summer seasons. An increasing trend in TVBN values (49 to 62.07, 106.4 to 110.6 and 55.53 to 63 mg100g⁻¹ for H. toli, H. nehereus and J. Caruta respectively) for all three experimental fishes was documented in monsoon and summer seasons (Table 3). This is important to note that, the temperature in monsoon and summer seasons was almost similar (Table 1). The increasing trends in TVBN have been reported in several other dried fishes in earlier works. In present study, this increments in TVBN values may be due to the fact that dried fishes are mainly kept for long in exposed or in open condition at ambient temperature which may absorb moisture from the atmosphere resulted in the increase of microbial load and in turn, this led to the increase in TVBN. However, this surge may also be due to post drying handling and transportation of the dried fishes. Higher relative humidity throughout the seasons supports the growth of microorganism yields increased TVBN values. A significant (p< 0.05)and positive correlation (r) between relative humidity vs TVBN values for H.toli, H. nehereus and J.Caruta were 0.76, 0.95 and 0.92 respectively during all whole seasons. However, in winter season TVBN values found to be decreased in all the cases may be attributed to lower temperature (average 24.62 °C) during season.

5.2.3 Changes in TMA in dried H. toli, H. nehereus, and J. caruta in different Seasons

TMAO is one the important compound found in the marine fishes. The reduction TMAO by bacterial activity and endogenous enzymes leads to the formation of TMA in fish. TMA is taken one of the important chemical quality parameters in fish analysis. The acceptable of TMA value is 50 mg% for dried fish for human consumption Spineli (1982). However, in case of fresh fish, suggested value is 10-15 mg/100 g Connell (1980) [9]. In the present study, none of the experimental species reached beyond 50 mg% in monsoon, winter or summers seasons (Table 3). Hence, as per TMA values, experimental dried fishes found to be fit for human consumption in all seasons. It also studied the dried fishes for TMA contents and revealed the values within the upper limit of human consumption. However, the findings of this study showed that TMA content of dried fishes was higher in monsoon season compared to summer and winter may be due the increase of spoilage bacteria level or bacterial count. The higher relative humidity and increased temperature in monsoon and summer seasons favours the bacterial and enzymatic activity results in TMA increment in foresaid seasons. The sudden increase of TMA-N and TVB-N in monsoon and summer season in the present investigation, are concurrent with the onset the bacterial putrefaction.

5.2.4 Changes in NPN in dried H. toli, H. nehereus, and J. caruta in different Seasons

The mean NPN content of three dried fishes viz. H. toli, H. nehereus, J. Caruta were 1.22, 1.34 and 1.23% respectively during all three seasons (Table 3). Dried fish samples collected from monsoon season shows high level of non-protein nitrogen. Similarly, Karthikeyan et al. (2007) [25] also observed NPN values in dried fish and that was almost identical range (0.9 to 2.5 %) to our experimental fishes. The high level of non-protein nitrogen in dried fish might be attributed to the variety of chemical reactions and break down of protein during the drying process. In our investigation, the NPN values found to have an increasing trends in monsoon and summer seasons while decreased in winter season. Here, this is important to note that the protein and NPN values having a negative and significant (p< 0.05) correlation values (-0.71, -0.89 and -0.73 for H.toli, H. nehereus and J. Caruta respectively) during three seasons. This negative correlation between protein and NPN indicate that the protein was degraded by bacterial and enzymatic activity in monsoon and summer months hence given rise to NPN.

5.2.5 Changes in peroxide value in dried H. toli, H. nehereus and J. caruta in different seasons

Peroxide value (PV) is the indicator of primary oxidation of lipids. PV values for dried H. toli, H. nehereus, J. Caruta are presented in Table 3. The findings of this study showed that the higher value of PV was found in dried H. toli compared to other two dried fish. It may be due to H.toli is fattier fish compared to other two experimental species which makes it susceptible for oxidation during drying and retailling. In addition to this, salting before drying of H. toil was done that is way may also have pro-oxidantive effect. In general, dried fish are likely to have higher PV values as they are exposed mostly in direct sunlight for longer time that may trigger the oxidation process. In the present study, the values of all three experimental dried fishes are higher in all three seasons (Table 3). The acceptable limit for PV is i.e. 20 millimoles of oxygen/kg of fish fat. Similar higher PV have been reported in dried fishes by several studies (Nath and Majumdar 2013; Kakati and Goswami 2013) [28, 23]. The PV for the three experimental species was increasing in summer and monsoon seasons and decreased significantly in winter season. This may be due to the fact that the temperature was higher in summer (29.29°C) and monsoon (29.40°C) season compared to winter season (24.62 °C). Moreover, it is important to note that temperature plays an important role in fat oxidation. The negative correlation between lipid content and peroxide value was observed for all three species. This indicates that there is decomposition of lipids and yields peroxides.

5.2.6 TBARS values in dried H. toli, H. nehereus, and J. caruta in different seasons

TBARS value is widely used as an indicator of the degree of secondary lipid oxidation in fish chemical analysis. TBARS number less than 3.0 mg malonaldehyde kg⁻¹ in cured fish is considered as acceptable limit. In the present study, the values for all the three experimental fish were lower than 3.0 mg malonaldehyde kg⁻¹ in monsoon, winter and summer seasons (Table 3). This indicates that the experimental fishes were fit for human consumption all seasons. However, Bhattacharya et al. (2016) [6] reported higher TBARS content (3.14- 6.27mg MDA/kg) in dried Bombay duck compared to present study. Similarly, Gok et al. (2008) [15] documented a wide range of...
TBARS i.e. 0.90 to 2.80 mg malonaldehyde/kg in dried fish. In this present study, TBARS value of dried fishes were higher in monsoon season may be due to exposure of the product during retail sale and effect of pro-oxidant like salt and metal and all three dry fishes were made by traditionally sun drying, it may leads of more degradation of peroxides during drying. The dried H. toli showed higher TBARS value compared to other two dry fish which may be due more peroxides formation due to salting and higher lipid content. A strong and significant (p< 0.05) positive correlation was found for three experimental species in all the seasons. This indicates that there was a strong degradation of peroxides leads to formation of secondary oxidation products such aldehyde, ketones and alcohols etc.

5.2.7 Changes in free fatty acids values in dried H. toli, H. nehereus and J. caruta during different seasons
Fish oil is reported to spoil very fast compared to other oils due to high amount of unsaturated fatty acids. The greater the degree of unsaturation, the greater would be chances of fat oxidation that results in causing rancidity in fat. It has been reported that there might be high risks of rancidity during prolonged storage conditions due to the fatty nature of fish Sohn and Ohshima (2011) [13]. In the present study, H. toil had higher FFA value that that of H. nehereus, J. Carata (Table 3). Overall, the FFA content for three experimental fishes were higher than that of reported for solar dried fish (0.02%) Immaculate et al. (2013) [21]. However, FFA contents similar to our study also reported in pre-dried fish and artificial dried products. Huss (1998) [19] suggested that high level of FFA is an indication of microbial spoilage activity.
In this present study, FFA values of dried fishes were higher in monsoon season may be due to hydrolysis of fats along with increase in microbial load as also reflected in PV and TBARS values. Boran et al. (2006) [7] reported that FFA is a measure of hydrolytic rancidity, the extent of lipid hydrolysis by lipase action and that fish oil containing high levels of polyunsaturated fatty acids, is very susceptible to oxidative deterioration at varying velocities, strongly depending on the storage conditions and fatty acid profile.

5.3 Microbiological quality of dried fishes
5.3.1 Changes in TPC in dried H. toli, H. nehereus and J. caruta during different seasons
The acceptable limit of bacterial load in dry fish is 5 log cfu g⁻¹ as suggested by ICMSF (1986) [20]. In present study, TPC of experimental dried fishes were higher and close to 5log cfu g⁻¹ in monsoon season (Table 4). Similarly, higher TPC in dried sardine species was recorded in monsoon season Prakash et al. (2011) [29]. This may be due to fact that dried fishes here in Agartala retail market are displayed in open in higher humidity (84.43 %) coupled with temperature (29.40°C) for longer period. The higher temperature and humidity are very conducive platform for microbial growth. Interestingly, in case of J. caruta, there was no significant (p< 0.05) change found in TPC during all three seasons of study year (Table 4). In case of H. toli and H. nehereus also no significant (p< 0.05) changes were observed in summer season. However, in winter season, TPC values for H. toli and H. nehereus slightly decreased from 4.86 to 3.81log cfu/g and 5.01 to 4.55 log cfu/g respectively (Table 4). The TPC range reported for sun dried fish and oven dried fish Gondotra et al. (2017) [14] was very wide but values reported by Kakati et al. (2017) [24] in dried fishes were similar to our findings. In the present findings, the r values for relative humidity vs TPC for H. toli, H. nehereus, J. Carata were 0.95, 0.92 and 0.94 respectively during three seasons. This positive and significant (p< 0.05) correlation between relative humidity and TPC indicates that higher relative humidity directly supports the microbial growth in dried fishes.

5.3.2 Changes in TFC in dried H. toli, H. nehereus and J. caruta during different seasons
In the present study, the dry fish samples were free from visible fungal colonies during winter and summer seasons whereas it was visible during monsoon season especially on H. toil (Table 4). Similar visible fungal colonies on salted and sun dried Sea Foods was reported in Tuticorin dry fish market in monsoon season Prakash et al. (2011) [29]. Nath and Majumdar (2013) [28] recorded by and large similar TFC values in market sample of dried P. sop bore and M. gulio. Overall, the fungal count were higher in H. toli compared to H. nehereus and J. carata. The TFC values were higher in monsoon season followed by winter and summer seasons (Table 4).This may be due to fact that dried fishes here in Agartala retail market are displayed in open, having higher humidity (84.43 %) coupled with temperature (29.40°C). The higher temperature and humidity are very ideal platform for microbial growth. In the present findings, the r values for relative humidity vs TFC for H. toli, H. nehereus & J. Carata 0.95, 0.94 and 0.94 respectively during three seasons. This significant (p< 0.05) positive correlation between relative humidity and TFC indicates higher humidity inspires the growth of fungus colonies in dried fishes.

5.3.3 Changes in total coliform and faecal coliform in dried H. toli, H. nehereus and J. caruta during different seasons
In the present investigation, the total coliform were higher in H. nehereus followed by H. toli and J. cuarta in monsoon season (Table 4). Similar result shown by Immaculate et al. (2013) [21] who revealed that higher MPN in monsoon (MPN 15-45/100 ml), followed by post monsoon season (MPN 7-20/100 ml) summer season (MPN 6 - 20/100 ml). In this present study, total coliform of dried fishes were higher in monsoon season may be due to higher moisture content leads to contamination of dried fishes, and also unhygienic practices followed during processing and drying process. The faecal coliform and Salmonella were totally absent in three dried fishes H. toli, H. nehereus, J. carata) in different season (Table 4). Faecal coliform and Salmonella both are pathogenic bacteria which contaminate foods from the external sources. However, in this present study, all three dried fishes were found free from faecal coliform and salmonella that indicates good handling practices undertaken in Tripura market. It is important to note that dry fish markets in Agartala are having raised platform for dry fish keeping and relatively hygienic. The absence of the pathogenic strains of faecal coliform and salmonella in experimental fishes revealed that these dried fishes are fit for safe human consumption in all the seasons.
### Table 1: Relative humidity and temperature during different seasons

<table>
<thead>
<tr>
<th>Months of sampling</th>
<th>June</th>
<th>July</th>
<th>August</th>
<th>September</th>
<th>October</th>
<th>November</th>
<th>December</th>
<th>January</th>
<th>February</th>
<th>March</th>
<th>April</th>
<th>May</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average Relative Humidity</td>
<td>81.62±7.26</td>
<td>85.74±8.08</td>
<td>86.45±8.18</td>
<td>85.40±7.51</td>
<td>81.41±6.62</td>
<td>73.23±7.85</td>
<td>72.64±9.76</td>
<td>68.38±8.98</td>
<td>63.32±10.74</td>
<td>61.03±11.61</td>
<td>68.90±7.14</td>
<td>70.22±7.62</td>
</tr>
<tr>
<td>Average Temperature (%)</td>
<td>28.73±3.79</td>
<td>29.44±2.84</td>
<td>30.31±2.31</td>
<td>29.13±2.96</td>
<td>27.28±1.82</td>
<td>25.66±3.01</td>
<td>24.34±0.94</td>
<td>20.51±1.63</td>
<td>25.12±1.84</td>
<td>28.10±0.97</td>
<td>29.43±1.48</td>
<td>30.34±1.71</td>
</tr>
</tbody>
</table>

Values presented in the table are means ± SE n= 30

### Table 2: Proximate composition of dried fishes (H. toli, H. nehereus, and J. Caruta) in different seasons

<table>
<thead>
<tr>
<th>Months of sampling</th>
<th>June</th>
<th>July</th>
<th>August</th>
<th>September</th>
<th>October</th>
<th>November</th>
<th>December</th>
<th>January</th>
<th>February</th>
<th>March</th>
<th>April</th>
<th>May</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (%)</td>
<td>47.31±0.06</td>
<td>44.62±1.01</td>
<td>43.57±1.06</td>
<td>42.11±0.52</td>
<td>42.35±1.00</td>
<td>42.93±0.06</td>
<td>43.22±1.03</td>
<td>44.10±1.06</td>
<td>44.68±0.06</td>
<td>46.55±1.00</td>
<td>46.49±0.06</td>
<td>46.76±0.00</td>
</tr>
</tbody>
</table>

### Table 3: Biochemical composition of dried fishes (H. toli, H. nehereus, and J. Caruta) in different seasons

<table>
<thead>
<tr>
<th>Months of sampling</th>
<th>June</th>
<th>July</th>
<th>August</th>
<th>September</th>
<th>October</th>
<th>November</th>
<th>December</th>
<th>January</th>
<th>February</th>
<th>March</th>
<th>April</th>
<th>May</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>5.9±0.09</td>
<td>6.3±0.18</td>
<td>6.7±0.18</td>
<td>6.9±0.18</td>
<td>6.4±0.10</td>
<td>6.4±0.10</td>
<td>6.4±0.10</td>
<td>6.3±0.19</td>
<td>6.4±0.10</td>
<td>6.4±0.10</td>
<td>6.3±0.19</td>
<td>6.4±0.10</td>
</tr>
</tbody>
</table>

### Table 4: Total number of fish in different seasons

<table>
<thead>
<tr>
<th>Months of sampling</th>
<th>June</th>
<th>July</th>
<th>August</th>
<th>September</th>
<th>October</th>
<th>November</th>
<th>December</th>
<th>January</th>
<th>February</th>
<th>March</th>
<th>April</th>
<th>May</th>
</tr>
</thead>
<tbody>
<tr>
<td>TVBN (mg 100g⁻¹)</td>
<td>49.4±9.2</td>
<td>53.2±0.81</td>
<td>58.3±0.81</td>
<td>62.0±1.07</td>
<td>60.6±0.47</td>
<td>57.4±0.81</td>
<td>53.2±0.48</td>
<td>50.4±0.81</td>
<td>46.6±1.23</td>
<td>43.4±0.81</td>
<td>45.2±0.47</td>
<td>47.1±0.33</td>
</tr>
</tbody>
</table>

### Table 5: Nitrogen content in different seasons

<table>
<thead>
<tr>
<th>Months of sampling</th>
<th>June</th>
<th>July</th>
<th>August</th>
<th>September</th>
<th>October</th>
<th>November</th>
<th>December</th>
<th>January</th>
<th>February</th>
<th>March</th>
<th>April</th>
<th>May</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPN (%)</td>
<td>1.15±0.10</td>
<td>1.29±0.10</td>
<td>1.33±0.10</td>
<td>1.36±0.10</td>
<td>1.30±0.10</td>
<td>1.15±0.10</td>
<td>1.05±0.10</td>
<td>0.94±0.02</td>
<td>0.87±0.02</td>
<td>0.93±0.01</td>
<td>0.99±0.01</td>
<td>1.22±0.02</td>
</tr>
</tbody>
</table>
### Table 4: Microbiological quality of dried fishes (H. toli, H. nehereus, and J. Carata) in different seasons

<table>
<thead>
<tr>
<th>Months of sampling</th>
<th>Monsoon season</th>
<th>Winter season</th>
<th>Summer season</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total Plate Count (log cfu/g)</strong></td>
<td><strong>June</strong></td>
<td><strong>July</strong></td>
<td><strong>August</strong></td>
</tr>
<tr>
<td><strong>Hilsa toli</strong></td>
<td>4.85±0.01&lt;sup&gt;g&lt;/sup&gt;</td>
<td>4.87±0.02&lt;sup&gt;f&lt;/sup&gt;</td>
<td>4.91±0.01&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Harpadon nehereus</strong></td>
<td>4.92±0.02&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.96±0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.09±0.06&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Johnius carata</strong></td>
<td>4.82±0.01&lt;sup&gt;e&lt;/sup&gt;</td>
<td>4.96±0.02&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.91±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Total Fungal Count (log cfu/g)</strong></td>
<td>3.67±0.03&lt;sup&gt;k&lt;/sup&gt;</td>
<td>3.76±0.04&lt;sup&gt;i&lt;/sup&gt;</td>
<td>3.85±0.02&lt;sup&gt;j&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Hilsa toli</strong></td>
<td>3.59±0.03&lt;sup&gt;h&lt;/sup&gt;</td>
<td>3.64±0.03&lt;sup&gt;h&lt;/sup&gt;</td>
<td>3.81±0.01&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Harpadon nehereus</strong></td>
<td>3.58±0.02&lt;sup&gt;e&lt;/sup&gt;</td>
<td>3.64±0.02&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.79±0.02&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Johnius carata</strong></td>
<td>15</td>
<td>21</td>
<td>23</td>
</tr>
<tr>
<td><strong>Hilsa toli</strong></td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td><strong>Harpadon nehereus</strong></td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td><strong>Johnius carata</strong></td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td><strong>Salmonella</strong></td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
</tbody>
</table>

Values presented in the Table are means ± SE n= 3, p < 0.05. Values in the row are bearing unlike letters differs significantly.
6. Conclusion
The absence of the pathogenic strains of faecal coliform and salmonella in dried fish showed that dried fishes sold in Agartala market are fit for human consumption. However, fish found to have better quality in winter season compared to monsoon and summer season.

7. Acknowledgment
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8. References