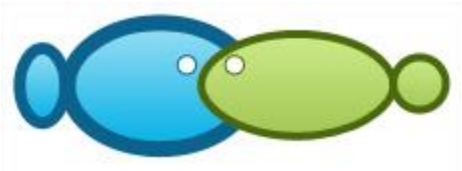

Biochemical, mineral compositions and microbial assessment of smoked hilsa (*Tenualosa ilisha*) with leaves, juice, and lemon peel

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Abstract. The current study was conducted to investigate the proximate minerals composition and microbial assessment of smoked hilsa (*Tenualosa ilisha*) with salt, lemon leaves extract, lemon juice and peel treatments under laboratory conditions. A total of 50 fresh hilsa fishes were collected from Meghna River, Bangladesh and were used for this study, with five treatments: T0 (control treatment, raw hilsa), T1 (8% salt), T2 (20% lemon leaves extract), T3 (20% lemon juice) and T4 (20% lemon peel with 8% salt). Proximate composition was analyzed, right after the smoking and 15 days later, for moisture, protein, fat, ash, calcium, magnesium, iron and phosphorous. It can be said that T3 (20% lemon juice) was the best among the four treatments. Smoked hilsa treated with salt and lemon juice may be a future food item for consumption in global markets.

Key Words: microbial assessment, minerals, proximate composition, smoked fish.

Introduction. As a tropical anadromous fish species, the hilsa shad, *Tenualosa ilisha*, is widely consumed and is considered as a popular traditional fish species in Bangladesh and is commonly known as Ilish from genus *Tenualosa* of the family Clupeidae, order Clupeiformes. Hilsa is the most important and biggest fishery resource of Bangladesh (Rahman et al 2020; Islam et al 2016). Hilsa is a migratory anadromous fish species which travels to the rivers from the sea during their breeding season to spawn in the Bangladeshi open water bodies, adjacent areas of India and after laying eggs in the rivers, they return to the sea. These newly hatched and juvenile hilsa used to stay in rivers for six to seven consecutive months before returning to the sea at maturity (Jahan et al 2015; Rashid et al 2019). Hilsa is a highly perishable fish due to ample content of essential fatty acids (Mohanty et al 2012). The supply of hilsa fish is not available in adequate quantity at any time around the year, only in certain seasons or months. Therefore, preservation of the hilsa fish is required to get year-round supplies for public consumption. There are numerous approaches accomplished for the preservation of hilsa fish products for later consumption which include icing, freezing, drying, salting, and smoking. Among these, smoked hilsa is a delectable fish product (Alam et al 2012; Hossain et al 2012; Debnath et al 2018; Saha et al 2015; Sarwar et al 2019). The process of smoking is considered as flavoring, heating, or storing food by exposing it to burning substances that smoke, utmost commonly wood and woody materials (Clucas & Ward 1996). Smoking has been more of a medium of flavoring than conserving foods, especially for different meats and fishes. Traditionally, smoking took place in a smoking

chamber heated to between 80 and 90 degrees Celsius. Burning wood vapor contains germicides and antioxidants that are absorbed by fish based on moisture levels and smoke streaming (Clucas & Ward 1996). The absorption rate of the ingredient varies among different aquatic animals including various fishes and, most significantly, fish has higher retention rate of germicides and antioxidants compared to shrimp because of higher moisture presence (Begum et al 2016b; Akuamoa et al 2018). The process of smoking can be carried out with garlic, coriander, and lemon to enhance the flavor as well as preservation quality. Some previous work analyzed the taste of smoked hilsa (Saha et al 2015); nutritious significance of smoked river and ocean hilsa (Debnath et al 2018); biochemical and minerals available in smoked hilsa treated with salt, garlic and coriander (Hossain et al 2012); nutritious substances and values of hilsa (Alam et al 2012); the impact of quality in hilsa food products by using different treating and conservation methods (Sarwar et al 2019); drying (Ahmed et al 1979; Morshed 2005), freezing and salting (Rabbane 2006; Sarmin 2008) however, no scientific research on the effect of lemon on smoked hilsa fish has been conducted. Hence, the purpose of the present work was to examine the functional properties of the smoked product of hilsa at various times after curing with leaves, juice, and lemon peel and to assess the microbial content.

Material and Method

Collection of fishes. A total 50 of fresh fishes were collected from the fish landing center at Chandpur, Bangladesh, which were caught from the Meghna River. The collected fishes were stored in an ice box and transported to the Institute of Food Science and Technology (IFST) laboratory of Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhanmondi, Dhaka for further analysis.

Experiment period. The study was carried out from July 2014 to March 2015.

Research design. The collected hilsa fishes were clustered into five groups where the first group composed of raw hilsa with no additives, second group comprised 8% salt concentration which was labeled as treatment 1 (T1). Other groups were, treatment 2 (T2) that contained 8% salt concentration with 20% lemon leaves, treatment 3 (T3) that 8% salt concentration with 20% lemon juice and treatment 4 (T4) that contained 8% salt concentration with 20% lemon peel (Table 1). A total of 10 replications for every treatment were considered for precision of data analysis.

Preparation of smoking. In the laboratory, the fishes were preserved at room temperature for a limited period to get into the normal condition. Then, the fishes were washed suitably with clean freshwater to eliminate the waste particle that was adhered with the body; then the internal organs were removed from every fish. The scales were removed from the fish skin carefully by using a descaling apparatus so that the skin was not cracked. After descaling, the fishes were rinsed with clean freshwater again. After that, the raw hilsa fishes were split diagonally into several number of small pieces with a sharp knife and washed with clean freshwater again. After washing, the split fishes were retained on a tray for a while to eliminate excess water; then, the fishes were weighed on a digital weight machine and separate into five groups for different treatments where 300 grams of fish was taken for each treatment (Table 1). Then the processed pieces of hilsa fish meat were treated with salt and other experimental ingredients. The control treatment was not treated with any ingredients; while the second treatment was treated with 24 g salt (8% of the sample weight) which referred treatment one (T1); fishes treated with 24 g salt and 60 g of lemon leaves extract which referred as treatment two (T2); fishes treated with 24 g salt and 60 ml of lemon juice considered as treatment three (T3); lastly treatment four contained 24 g salt and 60 g of lemon peel extract. After mixing all ingredients with the fish meat, they were placed on a tray for one hour for the salt and other ingredients to penetrate the muscular tissue, as well as to reduce the water content.

Table 1

Experimental design for processing of smoked hilsa

Treatments	*Weight of the raw fish (g)*	*Salt (8 % of the body weight) (g)*	*Lemon leaves* (g)*	*Lemon juice* (ml)*	*Lemon peel* (g)*
Raw Hilsa	300	0	0	0	0
Treatment 1	300	24	0	0	0
Treatment 2	300	24	60	0	0
Treatment 3	300	24	0	60	0
Treatment 4	300	24	0	0	60

* 20% of the body weight

Smoke curing. The fishes were anchored and arranged on racks suspended from the hoods; then the fire was constructed on level ground with non-sticky (locally available woods) wood chips and the temperature was maintained between 60-70^{0}C for 1-3 hours. The relative humidity was measured by using a hygrometer.

Storage. To avoid contamination the fishes were preserved by using polythene and were kept in refrigerator at 4^{0}C at all the time after the smoking process.

Mineral content and microbial assessment. The smoked hilsa were analyzed for mineral content and microbial assessment at day 0 and 15 days after the smoke process was finished. The biochemical, mineral compositions and microbial composition of smoked hilsa, such as moisture, salt content, ash percentage, TVN (Total Volatile Nitrogen), protein, fat, calcium, iron, magnesium, and phosphorus were determined based on AOAC (2005), Pearson (1976), Miller and Houghton (1945), Bligh and Dryer (1959), Vogel (1978), Fawcett and Wynn (1961) and Nordin (1976) works, respectively. Subsequently microbial assessment was conduct on each sample based on total viable count and total coliform analysis following AOAC (2005) protocols.

Microbial assessment of water. The microbial assessment of river water (the source river water, from where the fishes were harvested) for instance, Total Viable Count (TVC) total coliform, Staphylococci and Salmonella were measured and counted based on the work of Leong et al (2018), Khan et al (2017) and Ugbaja and Otokunefor (2015).

Statistical analysis. All statistical analysis were accomplished by using the SAS 9.4 software for Windows (version 9.4; SAS Institute Inc., 2012, Cary, NC, USA). Split-plot two-way analysis of variance (ANOVA) where $p < 0.001$ was applied on different treatments and various biochemical and mineral features to know the variations in different treatments with time. Tukey test was performed to acknowledge the significance level among different treatments.

Results

Biochemical composition of raw fish. The moisture content of raw fish varied roughly from 60 to 65, the protein content varied from 16 to 21, the fat percentage varied from 11 to 16, and the ash percentage varied from 1 to 2 %. The moisture percentage was 63.41±2.65%, protein was 18.69±1.38%, fat was 13.75±1.38% and the ash was 1.71±0.47%. The calcium percentage found was 46.76±3.62 mg/100g, magnesium was 0.37±0.15 mg/100g, iron was 10.88±0.88 mg/100g and phosphorous was 2.16±0.70 mg/100g in raw hilsa fish (Table 2). All values represent mean ± SD in Table 2.

Table 2

Biochemical composition of raw hilsa in the Fish Technology Laboratory, BCSIR, Dhaka

Parameters	*Value*
Moisture (%)	63.41±2.65
Protein (%)	18.69±1.38
Fat (%)	13.75±1.38
Ash (%)	1.71±0.47
Calcium (mg/100g)	46.76±3.62
Magnesium (mg/100g)	0.37±0.15
Iron (mg/100g)	10.88±0.88
Phosphorous (mg/100g)	2.16±0.70

Biochemical and mineral compositions of smoked hilsa in first analysis. In the first analysis (day 0), the moisture content of T3 (lemon juice) was significantly lowest (p<.0001) (25.29±1.37 %) among all four treatments, where T2 (lemon leaves) found the highest moisture content value (36.74±1.39%) (p<.0001) (Table 3). The significantly (p<.0001) highest (39.19±1.27%) protein percentage was found in T3 (lemon juice); whereas the lowest protein content were observed in T2 (lemon leaves) (27.55±1.38%) and other were T1 (35.25±1.41%) and T4 (lemon peel) (28.11±1.36%), respectively. Significantly highest (p<.0001) fat content (33.47±1.38%) was observed at T4 (lemon peel) whereas the lowest fat was reported in T3 (27.60±1.38%) and the other treatments were found with moderate fat contents for T1 (29.44±1.40%) and T2 (32.33±1.35%). The highest ash content (6.90±0.71%) was observed in T3 (lemon juice) which was significantly higher (p<.0001) among all other treatments and the lowest ash content was observed at T2 (lemon leaves) (2.46±0.70%). Ash content of other treatments were for T1 of 4.27±1.23 % and for T4 of 3.42±0.71 %, respectively. On the other hand, it was found that the calcium content (50.15±1.35 mg/100g), iron content (12.68±1.38 mg/100g), and phosphorus content (3.08±0.69 mg/100g) of treated hilsa in treatment 3 (lemon juice) observed significantly (p<.0001) higher values than other treatments. It was observed that the magnesium content of treated hilsa was moderately similar in all treatments (T1=0.40±0.09 mg/100g; T2=0.39±0.09 mg/100g; T3=0.38±0.10 mg/100g; T4=0.37±0.09 mg/100g) and there were no significant differences among treatments (<.0001). It was also observed that, there were no statistical differences between T4 and T2, in respect of phosphorus content in this first analysis of treated hilsa (Table 3).

Table 3

Biochemical and mineral compositions of smoked hilsa

Interval	*Features*	*T1*	*T2*	*T3*	*T4*	*p Value*
0 Days	Moisture (%)	$30.98±1.05^{c}$	$36.74±1.39^{a}$	$25.29±1.37^{d}$	$35.15±1.37^{b}$	<.0001
	Protein (%)	$35.25±1.41^{b}$	$27.55±1.38^{d}$	$39.19±1.27^{a}$	$28.11±1.36^{c}$	<.0001
	Fat (%)	$29.44±1.40^{c}$	$32.33±1.35^{b}$	$27.60±1.38^{d}$	$33.47±1.38^{a}$	<.0001
	Ash (%)	$4.27±1.23^{b}$	$2.46±0.70^{d}$	$6.90±0.71^{a}$	$3.42±0.71^{c}$	<.0001
	Calcium (mg/g)	$39.22±1.37^{c}$	$36.99±1.38^{d}$	$50.15±1.35^{a}$	$40.12±1.38^{b}$	<.0001
	Magnesium (mg/g)	$0.40±0.09^{a}$	$0.39±0.09^{a}$	$0.38±0.10^{a}$	$0.37±0.09^{a}$	<.0001
	Iron (Fe) (mg/100g)	$11.44±0.71^{bc}$	$11.77±1.38^{b}$	$12.68±1.38^{a}$	$11.34±1.39^{c}$	<.0001
	Phosphorous (mg/g)	$2.58±0.69^{b}$	$2.99±0.69^{ab}$	$3.08±0.69^{a}$	$3.00±0.69^{ab}$	<.0001
15 Days	Moisture (%)	$33.98±1.38^{c}$	$38.23±1.38^{a}$	$27.40±1.37^{d}$	$37.50±1.43^{b}$	<.0001
	Protein (%)	$36.02±1.36^{b}$	$27.02±1.39^{d}$	$38.89±1.36^{a}$	$27.94±1.38^{c}$	<.0001
	Fat (%)	$25.33±1.37^{d}$	$31.99±1.37^{b}$	$26.85±1.39^{c}$	$32.76±1.38^{a}$	<.0001

Ash (%)	4.00±0.71[b]	2.17±0.71[c]	6.20±1.69[a]	3.27±1.38[b]	<.0001
Calcium (mg/100g)	37.99±1.36[c]	36.63±1.38[d]	49.00±1.39[a]	39.680±1.38[b]	<.0001
Magnesium (mg/100g)	0.50±0.09[d]	0.60±0.09[b]	0.71±0.12[a]	0.58±0.09[c]	<.0001
Iron (Fe) (mg/100g)	9.26±3.01[b]	10.55±1.38[a][b]	11.39±1.38[a]	11.31±1.71[a]	0.0005
Phosphorous (mg/100g)	3.00±0.71[b]	3.29±0.70[ab]	3.59±1.38[a]	3.22±1.37[ab]	<.0001

Note: T – Treatment. All values represent mean ± SD. Different superscripts within the same column indicates significant differences (p<.0001 and p=0.0005).

Biochemical and mineral composition of smoked hilsa in second analysis. In the second analysis (day 15), the moisture content of smoked hilsa was found significantly (p<.0001) higher (38.23±1.38%) in T2 (lemon leaves) whereas lowest moisture content was found in T3 (lemon juice) (27.40±1.37%) and other treatments revealed the following values, T1 (33.98±1.38%) and T4 (37.50±1.43%) (Table 3). The protein content of T3 (lemon juice) was significantly (p<.0001) higher (38.89±1.36%) than other treatments (T1=36.02±1.36%; T4=27.94±1.38%); whereas in T2 we found the lowest (27.02±1.39%) protein value. However, the fat content of smoked hilsa in second analysis was found significantly higher in lemon peel T4 (32.76±1.38%) whereas the lowest fat content was observed in T1 (25.33±1.37%). The significantly (p<.0001) higher (6.20±1.69%) ash content was noticed in T3 whereas the lowest (2.17±0.71%) ash value was found in T2. On the other hand, calcium (49.00±1.39 mg/100g), magnesium (0.71±0.12 mg/100g) and phosphorus (3.59±1.38 mg/100g) content of T3 (lemon juice) found significantly (p<.0001) higher values than other treatments. Nevertheless, iron content of T3 (11.39±1.38 mg/100g) and T4 (lemon peel) (11.31±1.71 mg/100g) did not significantly (p=0.0005) differ among each other, but they were significantly different from T1 (9.26±3.01 mg/100g) and T2 (lemon leaves) (10.55±1.38 mg/100g) in the second analysis of smoked hilsa (Table 3).

Estimation of TVN (Total Volatile Nitrogen) and salt in the smoked fish. It was observed that, T3 (lemon juice) contained the lowest value of TVN; from where it can be easily understood that smoked hilsa treated with lemon juice had better condition than other; whereas T1 contained the highest percentage (6.86%) of salt and T2 (lemon leaves) showed the lowest (5.21%) salt content in the first analysis (Table 4). Whereas, the second analysis revealed that T3 contains the lowest value of TVN and however, salt content in second analysis was found higher (5.34%) in T3 compared to the other treatments.

Table 4

Average value of TVN and Salt in four treatments and their comparison

	0 Days (1st Analysis)		*After 15 days (2nd Analysis)*	
Treatments	*TVN (%)*	*Salt (%)*	*TVN (%)*	*Salt (%)*
T1	6.09	6.86	7.22	2.07
T2	7.04	5.21	7.70	4.80
T3	5.98	6.12	6.64	5.34
T4	6.41	6.67	6.82	5.02

Microbial assessment of smoked product. Microbial assessment of smoked hilsa revealed T3 (lemon juice) showed the best result in contrast of total viable microbial count (2.1×10^3); the other analysis such as, total coliform, and *Escherichia coli* found in an optimal edible rate where there was no *Salmonella* observed in smoked hilsa fish (Table 5).

Table 5

Microbial content in the smoke treated fish in laboratory condition

Parameters	*T1*	*T2*	*T3*	*T4*
Total Viable Count(TVC), (cfu/g)	2.3×10^3	2.1×10^4	2.1×10^3	2.0×10^4
Total Coliform, (MPN/g)	<3	<3	<3	<3
E. Coli, (MPN/g)	<3	<3	<3	<3
Salmonella/25g	Absent	Absent	Absent	Absent

Microbial assessment of source water sample. Experimental fishes were collected from Meghna River, Chandpur, Bangladesh. Microbial quality for the water sample of Meghna River was assessed at the Bangladesh Council of Science and Industrial Research (BCSIR), Dhaka (Table 6).

Table 6

Microbial content of water sample in laboratory condition

Parameters	*Amount*
Total Viable Bacterial Count (TBVC), (cfu/ml)	5.1×10^5
Total Coliform, (MPN/g)	2/100 ml
Staphylococci	125/100 ml
Salmonella	Absent

Discussion. The lower the moisture content of a processed food item, the more suitable it is for human consumption and the less susceptible it is to microorganisms and contaminations. Usually the higher the moisture content, the lower the nutrient content (Hossain et al 2014). Present study on the moisture content coincides with the findings of Begum et al (2016a), where they found 66.94 to 72.04 % of moisture in *Tenualosa ilisha*; Mansur et al (2018) found 68.05% moisture in raw hilsa (*Tenualosa ilisha*) at Cox's bazar; Hossain et al (2012) found 39.65±0.19% moisture in smoked hilsa; Debnath et al (2018) found 56.45 % of moisture in hilsa; while Begum et al (2016b) found 39.42 to 56.74% moisture in smoked hilsa; De et al (2019) found 41.02 to 75.06% moisture in different river systems in west Bengal, India; Hossain et al (2014) found 60.37% moisture in the hilsa muscle analyzed from the Bay of Bengal; Alam et al (2012) found 62.31 to 69.29% moisture in Godavari river hilsa. The amount of water in fish varies widely but in most cases the variation is between 70-80% (Nowsad 2007). The moisture percentage (%) for T1, T2 (lemon leaves), T3 (lemon juice) and T4 (lemon peel) varied roughly from 25.29 to 36.74% at first interval and from 27.40 to 38.23% at the second interval after smoking which coincides with the findings of different fishes, including hilsa, containing 35 to 45 % moisture, after 1-3 hours of hot smoking (Saha et al 2015). The data of 2nd analysis revealed that it got slight increase of moisture than first analysis data. After 1st analysis the fishes were stored in a refrigerator to preserve the quality from deterioration; as a result, some moisture was added during preservation, but when the second analysis was performed, it retained some moisture and revealed a slight increase in moisture. The present result showed that the percentage of moisture content of smoked Hilsa was less than 69.20% which was found by Nketsia and Sefa-Dedeh (2000). From the above result, it was observed that smoked hilsa treated with lemon juice found less moisture content than others in both treatments. Also, it can be said that after smoking the percentage of moisture had been reduced. As a result, the number of bacterial loads was lesser than the raw hilsa. Due to the less moisture percentage when the microbiological test was done it was found that

smoked hilsa treated with lemon juice contained fewer microorganisms than the other two treatments.

In raw fish, the protein percentage was found at 18.69% which was close to result of Begum et al (2016a) where they found 18.95 to 20.56 % of protein in raw fish; Mansur et al (2018) found 16.59% protein in raw hilsa at Cox's bazar; Hossain et al (2012) found 25.65% protein in smoked hilsa; Debnath et al (2018) found 15.98 % of protein in hilsa; Begum et al (2016b) found 20.06 to 31.01% protein in smoked hilsa; Alam et al (2012) found a protein parentage from 18.14 to 22.69 % in Godavari river hilsa. Whereas the protein percentage found is a bit higher in *Cirrhinus reba* (19.74%) and *Clarias gariepinus* (19.64%) by Osibona et al (2006). After smoking, the protein percentage content upsurged in smoked hilsa in comparison to raw hilsa and however during second analysis it slightly decreased. It was observed that the uppermost protein content was found in smoked hilsa treated with lemon juice. Swastawati (2004) also evaluated protein percentage upsurges from 20.30% (fresh fish) to 23.95% in case of milk fish (*Chanos chanos*) smoking.

Hilsa is considered as a high fat content fish; presence of HUFA (Highly Unsaturated Fatty Acids) and PUFA (Poly Unsaturated Fatty Acids) in hilsa made it a precious source of animal fat; while the organic modification by different plant extracts in T4 (lemon peel) showed the best result whereas T3 (lemon juice) showed the minimal fat content. This could have occurred due to the presence of a high concentration of citric acid in lemon juice, which degrades the HUFA and PUFA in hilsa. The fat composition of the experimental fishes were relatively similar with the study of Begum et al (2016a), in which found 4.97 to 8.21 % of fat in raw hilsa; Mansur et al (2018) found 13.52% fat content in raw hilsa at Cox's bazar; Hossain et al (2012) found 24.85% fat in smoked hilsa; Debnath et al (2018) found 16.18 % of fat content in hilsa; while Begum et al (2016b) found 16.12 to 12.47 % fat in smoked hilsa; Alam et al (2012) found 8.78 to 17.38 % of fat content in Godavari river hilsa. While the present result does not support the conclusions of Mazumder et al (2008) regarding *Gudusia chapra* (5.41%) due to difference in species, proximate composition, and habitat. Saha and Guha (1939) estimated proximate composition of 34 species, and they observed the highest amount of fat was in hilsa (19.4%) which was comparatively higher than some other available fishes which are considered as local species to Bangladesh for example *Heteropneustes fossilis*, *Channa striata* and *Ophiocephalus punctatus* where fat was assessed as 1.23%, 0.64% and 1.08%, respectively (Qudrat-i-Khuda et al 1962). Salam (2002) evaluated the uppermost fat content in *Heteropneustes fossilis* (3.25%) which is also less than the present study. Indian carp *Labeo bata* comprised of 4.67% fat (Sarower-E-Mahfuj et al 2012) which is also less than the present finding. It was observed that, the fat percentage of smoked hilsa is quite higher than raw product; but during the second analysis 15 days after smoking the fat percentage decreased as fat is a volatile substance. Fat composition of different fishes are also influenced by numerous ecological and biological factors for instance feed intake, maturation of gonads, spawning seasons of species, nourishments etc. Differences of seasons might affect the availability of food and interfere on the reproduction biology and create a significant impact on the biochemistry of tissue of the fishes, and principally fat (Bumb 1992).

The term "ash content" refers to the amount of ash in a food. The higher the amount of ash, the more fibrous the food is. The dissimilarity in ash content of hilsa may be accredited with the health condition and the availability of food in their respective feeding environment. The ash percentage of the raw hilsa was 1.71% which was closer to the result of Hossain et al (2014) who found hilsa muscles captured from Bay of Bengal and Arabian Gulf which comprised of 1.34% and 1.50% ash, respectively. Mazumder et al (2008) estimated ash content in *Ailia coila* as well as *Amblypharyngodon mola* which varied between 1.6% and 3.2%. Chukwu and Shaba (2009) assessed upper value of ash in *Clarias gariepinus* (3.06%) than the investigated hilsa fishes; while Begum et al (2016a) found 2.77 to 4.84 % of fat content in hilsa; Mansur et al (2018) found 3.19% ash in hilsa at Cox's bazar; Hossain et al (2012) assessed 3.50% ash in smoked hilsa; Debnath et al (2018) found 8.34 % of ash in hilsa; while Begum et al (2016b) found 3.09 to 4.19 % ash in smoked hilsa; whereas Alam et al (2012) found 0.73 to 1.68 % of ash

content in river hilsa of Godavari, India. The ash percentage for differently treated smoked hilsa increased after smoking which fluctuated from 1.35% (fresh fish) to 2.03 and 1.89% for two treatments (Swastawati 2004). The ash content of smoked hilsa treated with lemon juice is higher than the others for both time interval.

The concentration of minerals and trace element varies in fishes considering different influences for instance their feeding manners, environment, ecosystem, and migration (Andres et al 2000). Calcium (Ca) is an important mineral for the formation of bone in the body. The Ca amount in the current study of smoked hilsa is similar with the average value 144.21 to 372.67 mg/100g found by Begum et al (2016a). In another study Debnath et al (2018) reported 481.77mg/100g of calcium. Study of Alam et al (2012) found 204.12 mg/100 g of calcium in hilsa. Present study showed that smoked hilsa treated with lemon juice (T3) contains more Ca than others in both treatment and it is better for future consumption.

Magnesium (Mg) is an important mineral for the formation of bone in the body. The Mg value in raw hilsa and smoked hilsa was relatively similar; but in 2nd analysis (15 days after smoking) it increased slightly but is far lower than the value of 34.18 to 45.07 mg/100 g found by Begum et al (2016a). Study shows that smoked hilsa treated with lemon juice (T3) contains more magnesium than the other treatments and it is better for future consumption.

The iron (Fe) content of smoked hilsa in the present study was found to be similar with the values of 9.04-13.07% found by Begum et al (2016a). Study shows that smoked hilsa treated with lemon juice (T3) contains more iron than other treatments and it is better for future consumption.

The phosphorous (P) content in current assessment was found to be far less than the value of 118.17 to 204.06 mg/100g found by Begum et al (2016a). Debnath et al (2018) found 115.73 mg/100 g phosphorus in hilsa. Study shows that smoked hilsa treated with lemon juice (T3) contains more iron than the other treatments and it is better for future consumption.

TVN value indicates the condition of freshness of fish. The TVN value was evaluated to determine the condition of the fishes after the smoking. From the finding it can be clearly concluded that the fishes were good in taste and among the four treatments, smoked hilsa treated with lemon juice (T3) can be considered as most consumable hilsa product. Begum et al (2016a) found TVN value of 2.01 to 3.50 mg/100 g which is lower than average findings; Hossain et al (2012) found TVB-N value of 2.55 mg/100 g which is lower than present study. Where Mansur et al (2018) recorded highest value of TVN 23.39 mg/100 g because they took raw hilsa fish where the present study analyzed smoked hilsa.

As all four treatments contain salt (8% of body weight) and were kept for approximately one hour after salt was mixed with fish meat, it can be concluded that salt penetrates the body, but not uniformly. Salt also retards the growth of microorganisms in the fish body. Begum et al (2016a) found similar salt content result of 2.05 to 6.48 % in raw hilsa fishes; Hossain et al (2012) found 16.20% salt in garlic and coriander smoked hilsa fishes; Debnath et al (2018) found 1.92 ± 0.18% salt in riverine smoked hilsa fishes; Begum et al (2016a) revealed 1.02 to 5.27 to % of salt in lemon, mustard and garlic treated smoked hilsa fishes.

This experiment was also performed to observe the microbial loads in the treated fishes. The total coliform count of four treatment were <3MPN/g. *Salmonella* was absent in smoked hilsa (25 g). The TVC loads were near the result of Shamsuzzaman et al (2011), Sultana et al (2008) and Dutta et al (2018). Our objectives were to compare the treatments to see which give the best result, may be easily used to make the smoked product. Whereas, it can be said that smoked hilsa treated with lemon juice (T3) gave the best result as it was good in appearance, taste, flavor, less moisture percentage, lower TVN value, higher fat percentage and a lower number of microorganisms.

We performed the water microbial analysis and did not observe any *Salmonella*. This water microbial assessment found the first study in Chandpur point in Meghna River and other study does not revealed TBVC, TC, *Staphylococcus* etc. But in Northwest Borneo, Malaysia one study by Leong et al (2018) was conducted and they found 1.6 ×

10^4 to 3.0 × 10^4 cfu mL^{-1} of microbial load where the World Health Organization (WHO) standard was 1.0 × 10^2. High microbial load referred that the Chandpur point has huge organic load due to surrounding different factors, garbage throwing, and jute fermentation for instance. However, total coliform was found at 2 cell/100ml, where the WHO standard is 3 cell/100 ml; so, the water of Chandpur point of Meghna river can be considered as comparatively good water in microbial aspect.

Conclusions. This study revealed the nutritive quality of the smoked hilsa fish with salt, lemon leaves extract, lemon juice and lemon peel treatment and to identify the best one as a ready food item to eat. Additionally, the number of microorganisms that withstand these four treatments was determined. Thus, the best treatment was not based only on proximate composition, but microbial tests were also considered. 8% salt with 20% lemon juice was discovered to be the most effective composition for hilsa treatment during the smoking process. The high percentage of fat also indicated that the fishes comprised of good amount of n-3 and n-6 polyunsaturated fatty acids.

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