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*Research Article*

# Nutritional and microbiological assessment of *shidhal*, a semi-fermented fish product of Bangladesh

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ABSTRACT

The present study was conducted to evaluate the nutritional aspects (proximate composition, amino acid, Non-protein Nitrogen) and microbiological quality of the semi-fermented fish product locally known as *chepa shutki* or *shidhal*. Samples were collected from producers of Narsingdi and Mymensingh district as well as control sample were prepared with proper hygiene and sanitation in the laboratory. Statistical analysis showed significant differences ($p<0.05$) among the samples. The organoleptic quality of the laboratory prepared *shidhal* (LPS) and collected *shidhal* were evaluated on the basis of quality attributes such as color, odor, texture, insect infestation, overall quality and result showed that the overall quality of laboratory prepared *shidhal* was excellent whereas the samples from Narsingdi and Mymensingh were acceptable. The values obtained from proximate composition, NPN content and Total Plate Count (TPC) of bacteria of laboratory prepared *shidhal*, sample collected from Narsingdi and Mymensingh were moisture (31.24%, 33.71%, 37.22%), protein (43.91%, 42.62%, 42.73%), lipid (14.21% 14.62%, 6.54%), ash (12.19%, 11.05%, 12.90%), NPN (0.02%, 0.18%, 0.16%), TPC ($1.02\times10^5$, $1.96\times10^5$ and $1.96\times10^5$ CFU/g) respectively. Lower levels of protein, lipid and ash content in the product obtained from collected samples were probably related to the losses occurring at different stages of marketing chain during handling, transportation and preservation. Higher moisture content along with higher microbial load was found in the collected samples than control sample. Amino acid of collected and prepared *shidhal* were analyzed by HPLC and the highest percentages of total amino acids composition of laboratory prepared *shidhal*, sample collected from Narsingdi and Mymensingh were serine (3.25%, 3.46%, 11.35%), proline (1.59%, 0.94%, 0.002%), Phenylalanine (0.54%, 0.72%, 0.79%), Methionine (1.79%, 0.12%, 1.51%) respectively. The present study concludes that the overall nutritional and safety aspects of LPS was much higher than the sample collected from *shidhal* farmers indicating a lack of proper hygiene and safety measures.

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## 1. INTRODUCTION

Fisheries sector plays an important role in the national economy of Bangladesh. In Bangladesh total fish production is 42.76 lakh metric tons which provide 60% of the total animal protein supply in the diet of the people (DoF, 2019). This sector has great contribution in the economy of the country through providing 3.50% to the Gross Domestic Product (GDP) and 25.71% to the agricultural GDP (DoF, 2019). Fish and fishery products are highly nutritious, in addition to the high percentages of animal protein; they are good sources of some minerals like calcium, phosphorus and iron. A large number of people in Bangladesh suffer from various chronic malnutrition and most babies are born underweight since their mothers are chronically malnourished (Islam, 1998).

Traditional fishery products are native to a country or culture. Major traditional fishery products of Bangladesh include dried, semi-fermented, salted and some smoked products. *Shidhal* or *chepa shutki* is one of the major traditional fermented fishery products of Bangladesh where a large number of Bangladeshi people take it as traditional food item. It is a salt-free, solid, semi-fermented fish product, which is commonly consumed in all over the world, is prepared from a small sized fish mainly *Puntius sp*. and also known as *shidal, seedal, seepa, hidal* and *shidhal*. A large number of people from Mymensingh, Netrokona, Kishorgonj, Bhrammonbaria, Jamalpur, Tangail, greater Sylhet, Narsingdi and hilly regions of Chattogram are frequently involved with this semi-fermented product production and marketing and it plays a significant role by enhancing their socio-economic condition (Mansur, 2007). People prefer *chepa shutki* owing to its special taste and low-cost compared to other larger commercially important fish (Khanum *et al*. 1999; Mansur *et al*, 2000; Muzaddadi and Mahanta, 2013). *Shidhal* has unique magical aromatic power to attract consumers. Such attraction is never observed in any other food stuffs in Bangladesh. The World Health Organization (WHO) food safety unit has given high priority for the research in food fermentation, as it will improve the food safety by controlling the growth and activity of pathogens in foods. Moreover, fermented food products are a good source of peptides and amino acids. (Rajapakse *et al*, 2005, Sathivel *et al*, 2003). The calcium, phosphorus, magnesium and iron contents of chepa shutki were reported to be higher than those of similar kinds of Japanese processed fish and was regarded a high quality protein food (Khanum et al, 1999). Nahar *et al*. (2017) found 40% moisture, 34% protein, 18% lipid and 2% ash in chepa shutki. The calcium, phosphorus, magnesium and iron contents of chepa shutki were reported to be higher than those of similar kinds of Japanese processed fish and was regarded a high quality protein food. (Nahar *et al*, 2017). But the processors and workers do not follow regulation of sanitation and public health during preparation of it (Muzaddadi and Mahanta, 2013). Very often spoiled fishes are used to make it. Proper washing, transportation, preprocessing are not properly maintained. As a result, different kinds of bacteria such as, *Salmonella*, *E. coli*, *Shigella* can easily contaminate *shidhal* (Muzaddadi and Mahanta, 2013). Growth of fungus causes off flavors, soften the flesh and some can produce potentially dangerous mycotoxins under certain circumstances. So, determination of microbiological quality of such processed fishes is very important for guarding consumer's health and hygiene.

Moreover, as this semi-fermented fish product is only popular in some certain regions in Bangladesh, therefore there is a scope to disseminate this delicious item to whole country and can be selected as a medium of export to other countries, if it's nutritional quality and hygienic condition is properly maintained. So, this study is very important to improve socio-economic condition of the people of Bangladesh and to maintain safety of consumer. The present study has been carried out to assess the nutritional and microbiological quality of *shidhal* from different sources.

## 2. MATERIALS AND METHODS

### Collection of samples

Punti (*Puntius sophore*) were collected in fresh condition from Firingi Bazar, Karnaphuli fish market and Jhawtola Bazar, Chattogram from supplier in the early morning to prepare better quality *shidhal* in the laboratory. *Shidhal* were collected from Narsingdi and Mymensingh district to compare with the laboratory prepared *shidhal*.

### Sensory assessment of fresh fish

Sensory methods were used to assess the degree of freshness of raw punti (*P. sophore*) with the freshness grading scheme developed by Huss, (1988) in the laboratory.

### Preparation of *Shidahl* in laboratory

For the preparation of shidhal in the laboratory, at first good quality punti (*P. sophore*) were collected form the market then collection of abdominal oils in clean container by boiling the gut content. Then the fish were

washed with clean freshwater and sundried the fish for 5 days up to moisture content of 20-25%. After that dried punti were soaked in clean freshwater for 20 minutes then packed the fish into oil soaked vats by hand wearing gloves. Then the clay vat was sealed with polyethylene, lid and finally with clay and kept the clay vat in underground for six months.

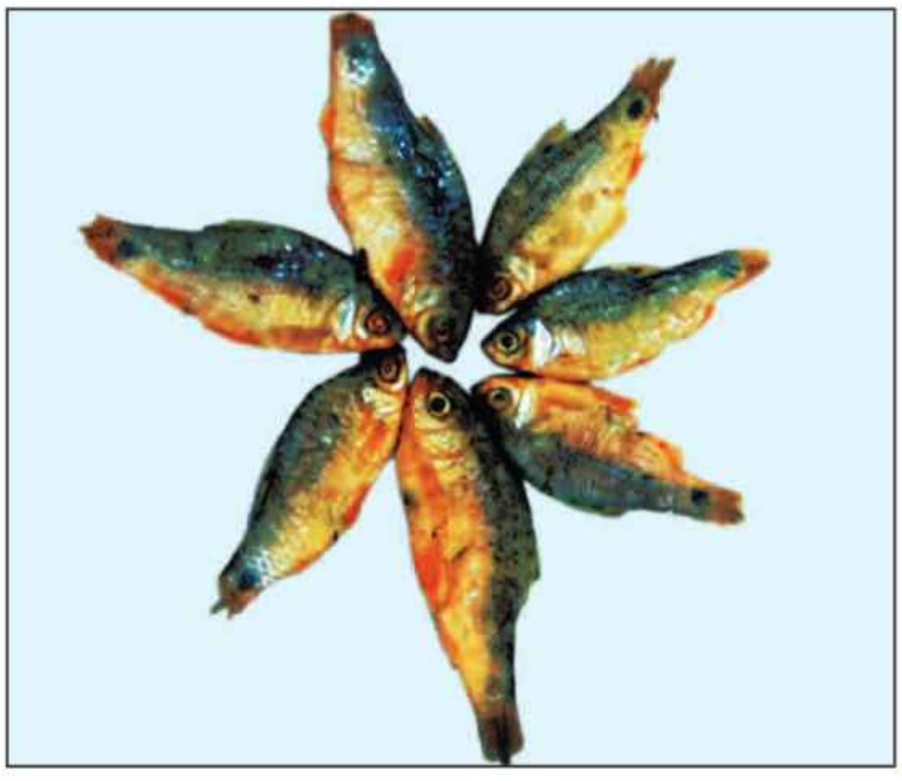

**Figure 1.** Laboratory Prepared *Shidhal*

### Quality assessment

Quality assessment of laboratory prepared *shidhal* and collected samples were carried out by sensory assessment, proximate analyses, amino acid analysis, Non-Protein Nitrogen (NPN) determination and microbiological analysis.

### Sensory assessment of smoked fish

The quality of the laboratory prepared *shidhal* and collected sample were assessed through organoleptic method described by Huss, (1988) based on color, appearance, taste, odor, texture, insect infestation and overall quality.

### Determination of nutritional composition of laboratory prepared and collected *shidhal*

Moisture, protein, ash, fat, amino acids and NPN content of laboratory prepared and collected *shidhal* were analyzed according to Association of Official Analytical Chemists (AOAC, 2016) methods with certain modifications.

### Microbial analysis of *shidhal*

Microbiological analysis ensures the quality of *shidhal*. For this reason, the total microbial load was determined by Total plate count (TPC) method by using plate count agar according to the method of AOAC, (2016) with certain modifications.

### Comparative study

Finally, a comparative study was carried out on the organoleptic, nutritional, chemical and microbial quality of laboratory prepared and collected *shidhal* to compare the quality of *shidhal*.

### Statistical analysis

The obtained data were stored in Microsoft Excel 2010 and the significance differences were examined through one-way analysis of variance (ANOVA) and Tukey multiple range tests using SPSS software version 21. The significance level was set at the level of $p<0.05$.

## 3. RESULTS AND DISCUSSION

### Organoleptic quality assessment

The organoleptic quality of the laboratory prepared *shidhal* and collected sample were assessed by sensory method (Huss, 1988) based on color, appearance, taste, odor, texture, insect infestation and overall quality. Results from the observations of physical and organoleptic characteristics of prepared and collected *shidhal* are presented in table 1. The color of the laboratory prepared *shidhal* and sample collected from Narsinghdi and Mymensingh were from slightly silver to slightly whitish in color. In laboratory prepared *shidhal*, the texture was firm and flexible but in marketed sample, the texture was slightly soft. There was no insect infestation in laboratory prepared *shidhal* and 1 month market sample. Some insect infestation was found in 12 months market samples. The overall quality of laboratory prepared *shidhal* was excellent and the sample collected from Narsingdi and Mymensingh were acceptable which indicates hygienic condition was maintained during preparation of *shidhal*. Nutritional composition, yield and consumers' acceptability of a ground semi-fermented fish product prepared from the underutilized fish species of the Bay of Bengal have been studied by Mansur *et al*, (2000). They prepared the product by a traditional semi-fermentation method, identical for the preparation of *shidhal* and was subsequently dried in oven, ground, packed in polyethylene bags and stored in ambient condition. Quality in terms of nutritional composition was found comparable to other fish products of Bangladesh and they could be able to show that the new product is equally acceptable.

**Table 1.** Organoleptic characteristics of laboratory prepared *shidhal* and collected sample

| Sample | Color | Order | Texture | Insect infestation | Overall quality |
|---|---|---|---|---|---|
| Laboratory prepared *shidhal* | Slightly yellowish | Characteristics odor | Firm and elastic | No infestation | Excellent |
| Sample collected form Narsingdi (1 month) | Slightly yellowish | Characteristics odor | Firm and elastic | No infestation | Acceptable |
| Sample collected form Narsingdi (12 month) | Slightly grayish | Characteristics odor | Firm and some loss of elastic | Some infestation | Acceptable |
| Sample collected form Mymensingh (1 month) | Slightly yellowish | Characteristics odor | Firm and elastic | No infestation | Acceptable |
| Sample collected form Mymensingh (12 month) | Slightly grayish | Characteristics odor | Firm and some loss of elastic | Some infestation | Acceptable |

**Nutritional profile analysis**

The proximate composition (moisture, protein, lipid and ash) and amino acid composition as nutritional profile of laboratory prepared *shidhal* and collected sample from Narsingdi and Mymensingh were analyzed by the Association of Official Analytical Chemists (AOAC, 2016) method with certain modifications.

**Proximate composition**

The proximate composition of Raw *P. sophore* and semi-fermented fish products, *shidhal* collected from Narsingdi, Mymensingh and laboratory prepared *shidhal* were analyzed and the results are presented and summarized in tabulated form.

**Moisture content**

Moisture content of raw *P. sophore* and laboratory prepared *shidhal* were in the range of 71.46±0.36% to 31.24±0.67% respectively. The results showed that there was reduction of moisture content after preparation of *shidhal* in the laboratory due to enzymatic degradation during fermentation. Moisture content of the collected sample of *shidhal* from Narsingdi, the moisture content varied from 33.71± 0.41% to 37.68±1.07% for 1 month and 12 months samples respectively. Higher moisture content was found in *shidhal* collected from Mymensingh which range from 37.22±0.70% to 41.73±0.06%. These values slightly differ from the values obtained by Nayeem *et al*, (2010) who found the proportion of moisture content 39.62%, 42.79% and 46.85%, respectively in the *Chepa Shutki* obtained from producer, wholesaler and retailer. This variation can be attributed to differences in period of maturation and the state of fish before preparation. Mansur, (2007) reported the higher percentage of moisture content in retailer sample indicates their improper handling and storage system. Similarly, some previous studies also revealed the similar outcome reported by Kakati and Goswami, (2013); Rahman, (2012). Statistical analysis revealed that moisture content was significantly ($p<0.05$) higher in samples collected from Mymensingh followed by Narsingdi and laboratory prepared *shidhal*.

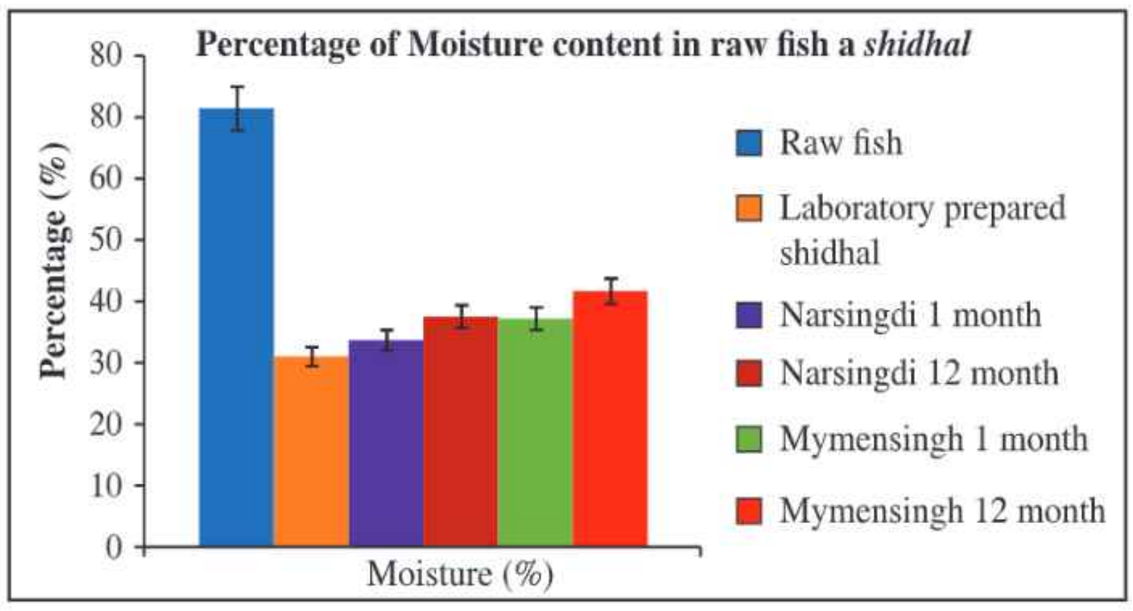


**Figure 2.** Percentage of moisture content in raw *P. sophore* and *shidhal* (Data are presented as Mean±S.D)

**Protein content**

The Protein content was varied from 20.32±0.56% to 43.91±0.97% in raw *P. sophore* and laboratory prepared *shidhal* on wet weight basis. For the collected sample of *shidhal* from Narsingdi, the protein content varied from 42.62±0.48% to 41.72±0.55% for 1 month and 12 months samples respectively. In case of *shidhal* collected from Mymensingh, protein content ranged from 42.73±0.53% to 41.57±0.77% for 1 month and 12 months sample respectively. Similar study was conducted by Nayeem *et al*, (2010) and they found the protein content of 33.83%, 32.78% and 32.46% respectively in the *Chepa Shutki* obtained from producer, wholesaler and retailer. Majumdar *et al*, (2016) reported that proximate analysis of protein content of chepa is 38.93% which were lower than that of the present finding because lower levels of protein in the product obtained from markets were probably related to the losses occurring at different stages of marketing chain during handling, transportation and preservation.

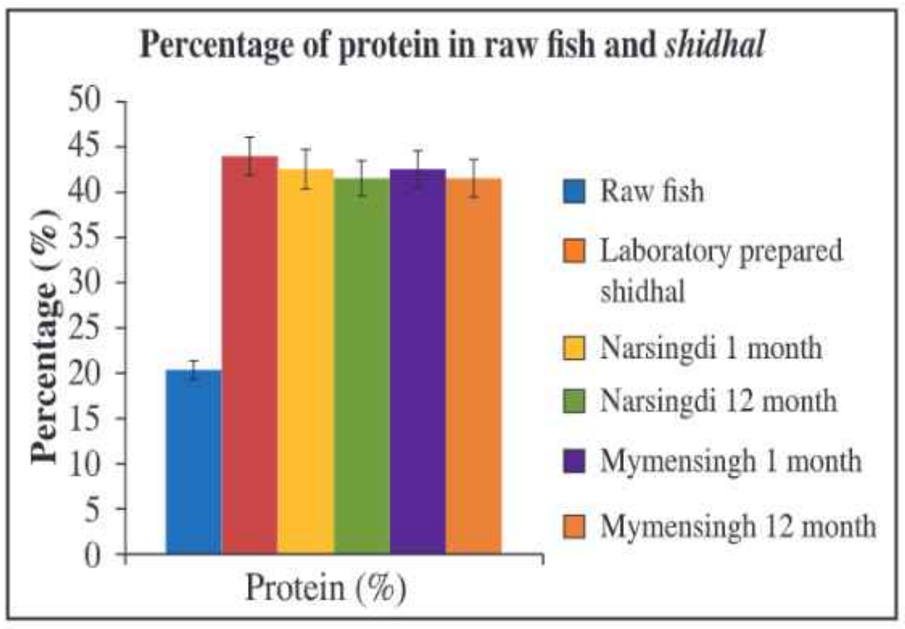


**Figure 3.** Percentage of protein content in raw *P. sophore* and *shidhal* (Data are presented as Mean±S.D

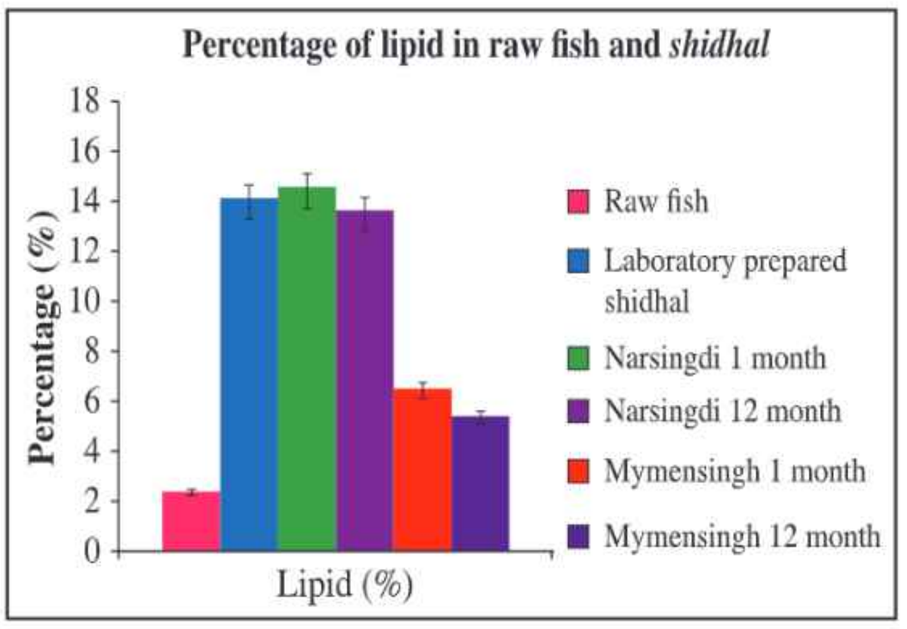


**Figure 4.** Percentage of lipid content in raw *P. sophore* and *shidhal* (Data are presented as Mean±S.D)

**Lipid content**

The lipid content was varied from 2.36±0.56% to 14.21±0.31% in raw *Puntius sophore* and laboratory prepared *shidhal* on wet weight basis. For the collected sample of *shidhal* from Narsingdi, the lipid content varied from 14.62±0.57% to 13.67±0.56% for 1 month and 12 months samples respectively. In case of shidhal collected from Mymensingh, lipid content ranges from 6.54±0.41% to 5.41±0.52% for 1 month and 12 months sample respectively. Similarly, Nayeem *et al*, (2010) got the proportion of lipid content as 24.97%, 22.47% and 19.25%, respectively in the Chepa shutki obtained from producer, wholesaler and retailer. However, significantly ($p<0.05$) highest lipid content was observed in sample collected from Mymensingh than sample collected from Narsinghdi. Majumdar *et al*, (2016) reported that lipid content of Chepa is 16.73% which significantly higher than the present finding. The increased rate of lipid content in the product is obvious due to reduction of moisture content and there is an inverse relationship with moisture content and fat content. Nayeem *et al*, (2010) reported that the poor content of lipid in *shidhal* is probably due to the loss of quality at different stage of marketing chain during handling, transportation and preservation.

**Ash content**

The ash content was varied from 5.44±0.31% to 12.19±0.71% in raw *P. sophore* and laboratory prepared *shidhal* on wet weight basis. For the collected sample of *shidhal* from Narsingdi, the ash content varied from 11.05±0.25% to 10.07±0.05% for 1 month and 12 months samples respectively. In case of *shidhal* collected from Mymensingh, ash content ranges from 12.90±0.20% to 11.16±0.16% for 1 month and 12 months sample respectively. Nayeem *et al*, (2010) observed that the ash content was 0.81%, 1.01% and 0.89%, respectively in the *Chepa Shutki* from producer, wholesaler and retailer. This agrees well with the findings of Ahmed *et al*, (2013) who reported that the average ash contents of producer and retailer sample were 2.26±0.04% and 2.37±0.02%, respectively. Ahmed *et al*, (2013) mentioned that the higher ash content noticed in the products sampled from retailers are probably associated with contamination with filth, sand, dust etc. which might occur during handling, transportation and preservation in the marketing chain.

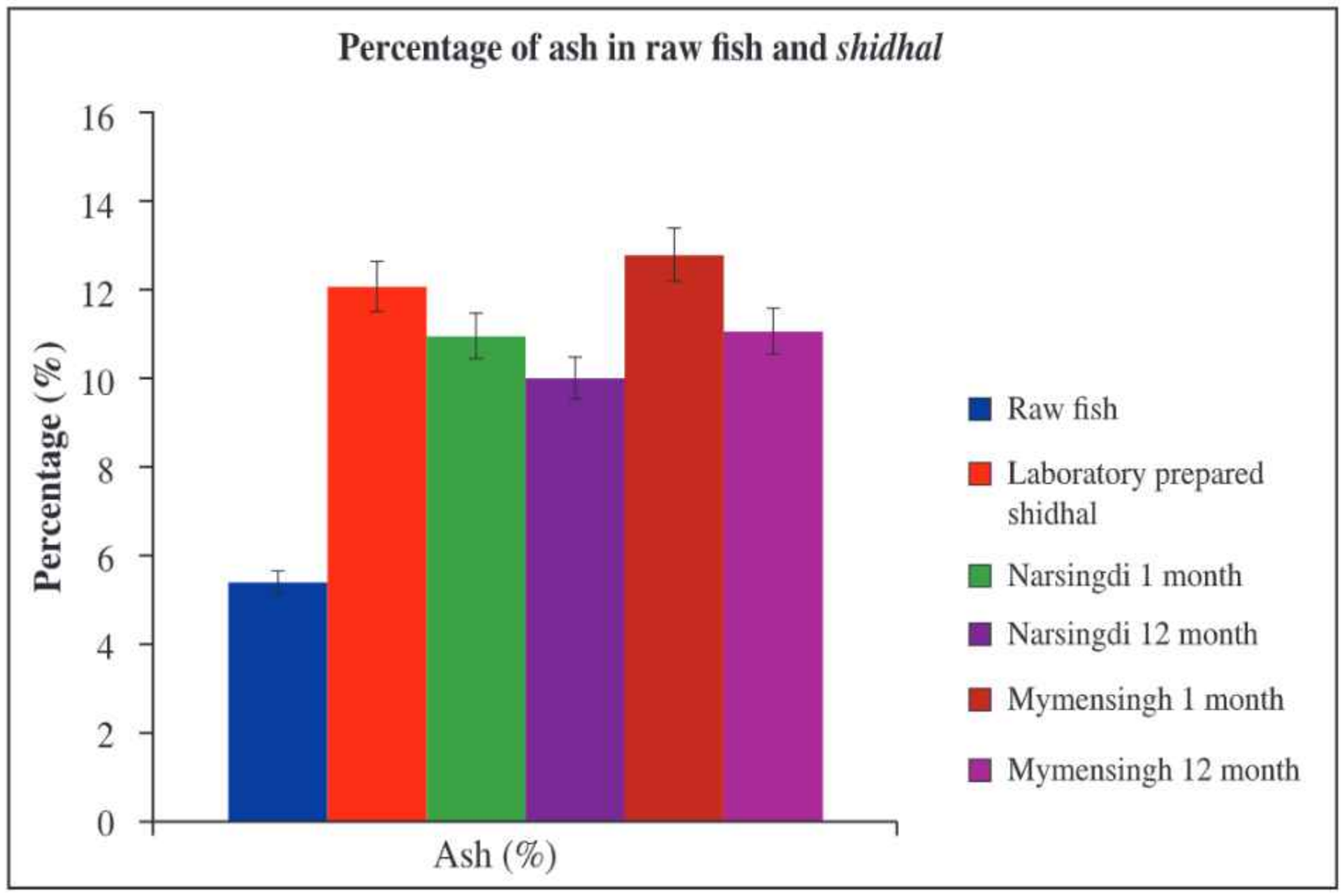


**Figure 5.** Percentage of ash content in raw *P. sophore* and *shidhal*.
(Data are presented as Mean±S.D)

**Table 2.** Proximate composition of raw *P. sophore* and *shidhal*

| Sample | Moisture (%) | Protein (%) | Lipid (%) | Ash (%) |
|---|---|---|---|---|
| Raw punti | $71.46^{a}$±0.36 | $20.32^{c}$±0.56 | $2.36^{c}$±0.56 | $5.44^{d}$±0.31 |
| Laboratory prepared shidhal | $31.24^{e}$±0.67 | $43.91^{a}$±0.97 | $14.21^{a}$±0.31 | $12.19^{a}$±0.71 |
| Narsingdi 1-month shidhal | $33.71^{d}$±0.41 | $42.62^{ab}$±0.48 | $14.62^{a}$±0.57 | $11.05^{b}$±0.25 |
| Narsingdi 12-month shidhal | $37.68^{c}$±1.07 | $41.72^{b}$±0.55 | $13.67^{a}$±0.56 | $10.07^{c}$±0.05 |
| Mymensingh 1-month shidhal | $37.22^{c}$±0.70 | $42.73^{ab}$±0.53 | $6.54^{b}$±0.41 | $12.90^{a}$±0.20 |
| Mymensingh 12-month shidhal | $41.73^{b}$±0.06 | $41.57^{b}$±0.77 | $5.41^{b}$±0.52 | $11.16^{b}$±0.16 |

***(The mean values having different superscripts differ significantly,Vertical bar =Mean ±S.D)

**Amino acid content of raw *P. sophore* and *shidhal***

The highest percentages in total amino acids of raw *P. sophore* were glutamic acid (2.16%), methionine (1.03%), arginine (0.85%) and serine (0.82%). In laboratory prepared *shidhal*, the highest percentages of total amino acids were serine (3.25%), proline (1.59%), aspartic acid (1.83%) and methionine (1.79%). The highest percentage of total amino acid in collected *shidhal* from Narsingdi and Mymensingh were serine (3.45% and 11.35%), valine (1.15% and 0.001%) and threonine (0.23% and 2.11%). Similar study was conducted by Majumdar *et al*, (2009) assessed the nutritional quality of *shidhal* and found glutamic acid (14.15%), aspartic acid (7.71%), leucine (6.81%), alanine (7.55%) and lysine (6.16%) in the higher amount and amino acids such as tyrosine (1.57%), histidine (1.11%), arginine (1.97%) and tryptophan (1.17%) have been found very low in amount.

**Table 3.** Percentage (%) of amino acid present in raw *P. sophore* and shidhal

| Components (%) | Raw *P. sophore* | Laboratory prepared *shidhal* | *Shidhal* collected from Narsingdi | *Shidhal* collected from Mymensingh |
|---|---|---|---|---|
| **Essential amino acids** | | | | |
| Lysine (Lys) | 0.78± 0.03 | 0.20±0.01 | 0.15±0.05 | 0.13±0.01 |
| Histidine (His) | Absent | 0.68±0.09 | 0.41±0.09 | Absent |
| Arginine (Arg) | 0.85±0.071 | 0.004±0.001 | 0.002±0.001 | 0.02±0.01 |
| Threonine (Thr) | Absent | Absent | 0.23±0.01 | 2.11±0.15 |
| Valine (Val) | 0.01±0.0007 | 1.60±0.37 | 1.15±0.08 | 0.001±0.001 |
| Methionine (Met) | 1.03±0.03 | 1.79±0.01 | 0.12±0.01 | 1.51±0.03 |
| Isoleucine (Ile) | 0.002±0.01 | 0.02±0.01 | 0.01±0.001 | 0.001±0.0001 |
| Leucine (Leu) | 0.01±0.01 | 0.35±0.01 | 0.35±0.03 | 0.43±0.01 |
| Phenylalanine (Phe) | 0.48±0.01 | 0.54±0.62 | 0.72±0.01 | 0.79±0.02 |
| **Non-essential amino acids** | | | | |
| Aspartic acid (Asp) | 0.47±0.03 | 1.83±0.08 | 1.37±0.03 | 0.60±0.03 |
| Serine (Ser) | 0.82±0.06 | 3.25±0.03 | 3.46±0.53 | 11.35±0.37 |
| Glutamic acid (Glu) | 2.16±0.06 | 0.01±0.01 | Absent | Absent |
| Proline (Pro) | 0.38±0.01 | 1.59±0.02 | 0.94±0.01 | 0.002±0.001 |
| Glycine (Gly) | Absent | Absent | 0.001±0.001 | 0.001±0.0001 |
| Alanine (Ala) | 0.07±0.01 | 0.01±0.001 | 0.01±0.001 | 0.01±0.001 |
| Cystine (Cys) | 0.004±.001 | 0.07±0.01 | 0.03±0.02 | 0.01±0.001 |
| Tyrosine (Tyr) | 0.01±0.01 | 1.12±0.15 | 0.93±0.06 | 0.38±0.01 |

***(The mean values having different superscripts differ significantly,Vertical bar =Mean ±S.D)

**Non-Protein Nitrogen (NPN) content and microbiological assessment of raw *P. sophore* and *shidhal***

The values of non-protein nitrogen (NPN) of raw fish, laboratory prepared *shidhal* and collected *shidhal* were assessed to evaluate the quality of *shidhal*. The NPN values of the raw fish and laboratory prepared *shidhal* were (0.01±0.006% and 0.02±0.003%) respectively. For the collected sample of *shidhal* from Narsingdi, the NPN content varied from 0.18±0.03% to 0.38±0.05% for 1 month and 12 months samples respectively. In case of *shidhal* collected from Mymensingh, NPN content ranged from 0.16±0.04% to 0.39±0.02% for 1 month and 12 months sample respectively.

In case of collected sample, the study showed that NPN content was higher in 12 months sample than 1-month sample because of increase in microbial decomposition. Thippeswamy *et al*. (2001) reported that the high level of non-protein nitrogen in fish might be attributed to the variety of chemical reactions and break down of protein.

The microbiological quality assessment of raw *P. sophore*, laboratory prepared *shidhal*, sample collected from Narsingdi and Mymensingh were carried out to compare the microbiological quality of laboratory prepared *shidhal* and collected sample. Results showed that microbial load in raw *P. sophore* and laboratory prepared *shidhal* were 1.14±0.07×10$^5$cfu/g and 1.02± 0.03×10$^5$ cfu/g respectively. *Shidhal* collected from Narsingdi and Mymensingh showed microbial loads of (1.96±0.06×10$^5$ and 3.09±0.13×10$^5$) cfu/g and (1.96± 0.08×10$^5$ and 3.09±0.78×10$^5$) cfu/g. In case of laboratory prepared *shidhal*, total plate count of bacteria was lower than market sample which indicates that the quality of fishes was good and hygienic condition were properly maintained during preparation of *shidhal* in the laboratory. A similar study was conducted by Ahmed *et al*, (2013) to analyse the proximate composition and bacteriological analyses of *shidhal* sample from producers as well as retailers of eight different districts of Assam and found promising microbial load in the retailer's

samples reflected poor quality, whereas those obtained in producer's samples were within the acceptable limit. Muzaddadi, (2002) also found that TPC value were around 4 log cfu $g^{-1}$ in chepa. Another similar study was conducted by Nahar *et al*, (2017) to analyse the proximate composition and bacteriological analyses of *Shidhal* and observed that the total bacterial count of *chepa shutki* ranged from 6.01±0.28 to 8.10± 0.18 log cfu $g^{-1}$ with the highest value in retailer's samples, while the lowest value was reported in control samples.

**Table 4.** Non-Protein Nitrogen (NPN) content and microbiological assessment of raw *P. sophore* and *shidhal*

| Sample type | NPN (%) | TPC (cfu/g) |
|---|---|---|
| Raw punti | $0.01^{c}$±0.006 | $(1.14±0.07× 105)^{c}$ |
| Laboratory prepared *shidhal* | $0.02^{c}$±0.003 | $(1.02±0.03× 105)^{a}$ |
| Narsingdi 1-month *shidhal* | $0.18^{b}$±0.03 | $(1.96±0.06× 105)^{b}$ |
| Narsingdi 12-month *shidhal* | $0.38^{a}$±0.05 | $(3.09±0.13× 105)^{a}$ |
| Mymensingh 1-month *shidhal* | $0.16^{b}$±0.04 | $(1.96±0.08× 105)^{b}$ |
| Mymensingh 12-month *shidhal* | $0.39^{a}$±0.02 | $(3.09±0.78× 105)^{a}$ |

***(The mean values having different superscripts differ significantly,Vertical bar =Mean ±S.D)

## 5. CONCLUSIONS

The present study was focused to compare the quality of semi-fermented *shidhal* prepared in the laboratory under controlled conditions and samples collected from different areas that are usually famous for *shidhal* production. From the nutritional and safety aspects, it was found that the LPS were superior to samples collected from different areas. It is therefore necessary to maintain the proper hygiene and sanitation in every stage of *shidhal* production to ensure a nutritionally enriched and safe product to the consumer.

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