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Comparisons of Human Pathogenic Microorganisms in Fresh and Stored '*Nappi*' (Fish-Paste) - An Ethnic Foodstuff of Bangladesh

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Abstract: *Nappi* is an ethnic fish-foodstuff made through fermentation. Microbiological comparison of two phases (fresh and one month stored) of *Nappi* were pragmatically assessed. The samples were collected from two different markets (Chaufaldandi and Harbang) of Cox's Bazar district from November 2013 to February 2014. Total Bacterial Count (TBC), Total Coliform (TC) and Fecal Coliform (FC), *Salmonella, Vibrio* spp. and yeasts and molds spp. were identified and counted from the collected samples. In the case of fresh *Nappi*, the higher densities of TBC (1.43×10^{10} CFU/g), TC (7.75×10^{9} CFU/g), FC ($5.87\pm0.11\times10^{9}$ CFU/g), *Salmonella* spp. ($2.6\pm0.10\times10^{7}$ CFU/g) and yeast and molds spp. ($5.05\pm0.16\times10^{6}$ CFU/g) were found in Harbang market and higher density of *Vibrio* spp. ($3.35\pm0.25\times10^{6}$ CFU/g) was in Chaufaldandi market. Whereas in stored *Nappi*, the higher densities of TBC ($1.63\pm.02\times10^{10}$ CFU/g), TC ($8.55\pm0.15\times10^{9}$ CFU/g), FC ($6.99\pm0.13\times10^{9}$ CFU/g), *Salmonella* ($9.5\pm0.30\times10^{7}$ CFU/g) *and Vibrio* spp. ($5.55\pm0.35\times10^{6}$ CFU /g) were found in Chaufaldandi and yeast and molds spp. ($6.85\pm0.15\times10^{6}$ CFU/g) were in Harbang market. *Nappi* might have been contaminated due to rough handling, unhygienic and poor preparation technique, poor transportation and improper storage. Although species level determination of the microbes was out of scope in this study, but this high number of colonies of *Vibrio* and *Salmonella* increase the probability of having pathogenic *V. cholerae, S. typhi* or *S. paratyphi* in *Nappi* which may cause dangerous diseases such as diarrhea, typhoid, paratyphoid etc.

Key words: *Nappi* · Ethnic People · Bacterial Density · Fresh · Stored · Pathogenic Species · Hygienic

INTRODUCTION

Fish and fishery products are consumed as nutritious food all over the world. The nutritional value of fish mostly depends on the freshness of fish. Initiation of spoilage starts reducing the nutritional value of fish. So processing of fish should be done as soon as possible. But most of the people of Cox's Bazar, Bangladesh have no refrigeration facilities. That is why they follow some preservation techniques such as rising temperature (e.g., boiling or frying); reducing the moisture content (by salting, smoking and drying); lowering the pH (by fermentation) which might cause changes to the flavor and texture of the fish and produce different fishery products [1]. *Nappi* is one of the most popular and traditional fermented fishery products which is made by most of the ethnic tribal community such as Marma, Tripura, Chakma and specially Rakhaing of hill districts (Chittagong and Chittagong hill tracts) and coastal area such as Cox's Bazar, Teknaf, Barguna and Patuakhalli in Bangladesh. Rakhaing are most popular *Nappi* maker and seller among the ethnic circle. *Nappi* is a fermented semi-solid fish-paste with potent flavor. It is an inexpensive source of protein for underprivileged and economically deprived ethnic community of Bangladesh. It is also very popular cuisine of our neighbouring countries and known as terasi in Indonesia, ngapi in Myanmar, kapi in Thailand, belacan in Malay, haam ha/ha jeung in Cantonese Chinese and homha\haeko in Min Nan Chinese [2].

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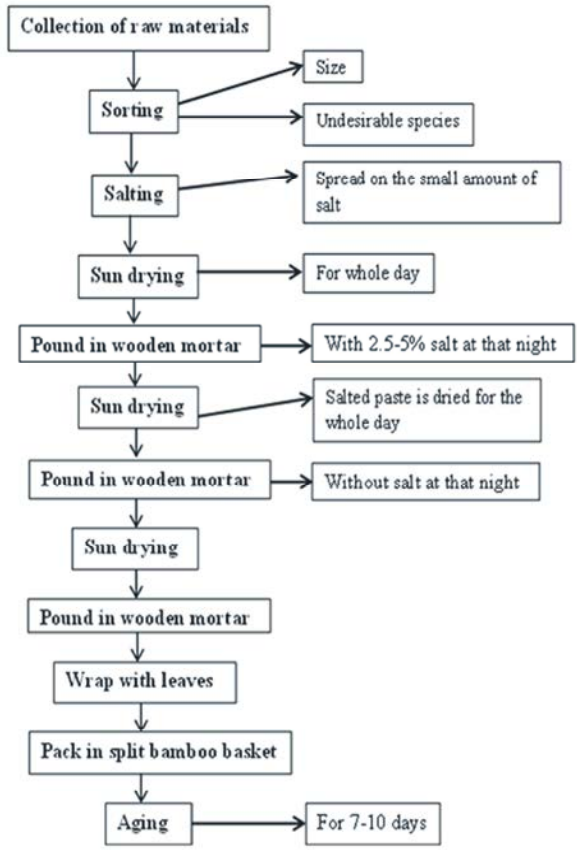

Fig. 1: Processing technique of traditional fermented product (*Nappi*).

Legend of Figure 1: The first step of processing technique of traditional fermented product (Nappi) was collection of raw materials and then these raw materials were sorted on the basis of size and undesirable species. Small amount of salt is spread over the mat and then shrimps are spread in thin layers over the mats. Shrimps on the mat are dried in the sun for the whole day. Semi dried shrimp is ground in wooden mortar with salt at the following night. On the following day, salt-ground paste is dried in the sun on the mat for the whole day and ground again at night. Salt is not added during second grinding. Similarly, the product is dried for the 3rd day and ground finally into paste at night with no further salt incorporation. The final paste has got a deep-grayish to blackish appearance. Final paste is shaped into block or globe and wrapped by large leaves of a wild hill tree called "mos-pata". Wrapped up Nappi is packaged in light basket made of split bamboo, locally called "khachi". Dough of 20 kg is packaged in one khachi and kept for 7-10 days for aging.

Mainly, the traditional fish and fishery products such as dried, salted, smoked, partially fermented and fermented (*Nappi*) are popular because of their characteristic color, flavor, taste; low cost (From the present study); long shelf life in edible form and long storage life without refrigeration for the fermentation process.

Typically, small shrimps such as *Acetes* and *Mysid* spp. are used to make *Nappi* in Cox's Bazar and Bandarban region. In Rakhaing villages, the whole process (Figure 1) is done under very unhygienic conditions and there might have chances of raw materials and finished products (From the present study). That is why it is vital to estimate the bacterial (TBC, TC, FC) and fungal load and to find out the incidence of *Vibrio* and *Salmonella* spp. in fresh and one month stored *Nappi*.

MATERIALS AND METHODS

Experimental Samples: The two phases of *Nappi* i.e. fresh and one month stored were separately examined for each specimen. The methods applying in this research work were followed from ICMSF [3].

Collection of Samples: The study area of this research was Cox's Bazar district of Bangladesh. The *Nappi* samples were collected in a fresh condition (Just after production) from the local market of Chaufaldandi union of Cox's Bazar Upazila because this area is important for producing *Nappi* abundantly and from Harbang union of Chakaria Upazila because this area is important for *Nappi* wholesaling and retailing (Table 1). The samples were collected in a sterilized plastic bag and later transferred in the laboratory refrigerator. And some *Nappi* samples were stored in laboratory in an open air without refrigeration from one month.

The study was conducted for a period of time from September 2013 to February 2014 in the laboratory of the Department of Fisheries and Marine Science, Noakhali Science and Technology University of Bangladesh.

Processing of Samples: Before processing of samples, All the glass wares such as conical flasks, beakers, measuring cylinder, test tubes, l-shaped glass rod, Petridish, inoculation tips etc. were washed, dried and sterilized in autoclave (40B series, LDZX) at a temperature of 121°C for 15 minutes at 15lb/inch^2 pressure.

Table 1: Sample size of *Nappi* collected from two different markets (Chaufaldandi and Harbang)

Samples	Source of samples	Sample size
Fresh *Nappi*	Chaufaldandi market (Cox's Bazar Upazila)	10
One month stored *Nappi*	Chaufaldandi market (Cox's Bazar Upazila)	10
Fresh *Nappi*	Harbang market (Chakaria Upazila)	10
One month stored *Nappi*	Harbang market (Chakaria Upazila)	10

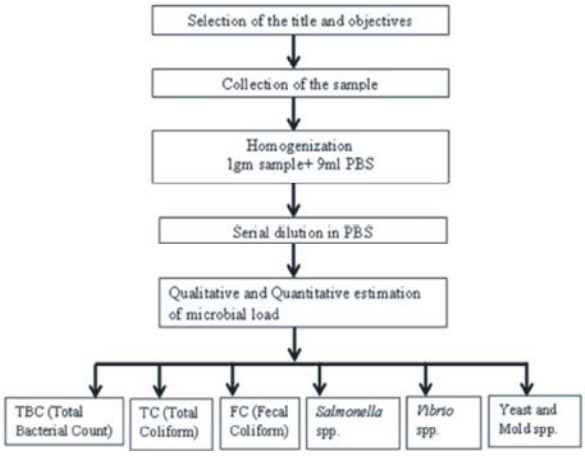

Fig. 2: Flow diagram of the methodology applied in this study.

Legend of Figure 2: The first step of this research work was selection of titles and objectives and then collection of sample Nappi from markets aseptically. Then the samples were homogenized separately with PBS solution. Then the dilutions of samples were made separately upto 10^{-5} by using serial dilution technique. l00µl from diluted solution of each sample were transferred to culture media containing petri-dish and inoculated using spread plate method for bacteriological analysis.

The samples were stored in refrigerator after collection and weighed with a sterile aluminum foil. The samples (1 gm of each sample) were homogenized separately with PBS solution. Then the dilutions of samples were made separately up to 10^{-5} by using serial dilution technique.

Culture media containing petri-dish was inoculated with l00µl of diluted solution of each sample using spread plate method [4] for enumeration of bacterial load. All the used media in this study was prepared media by Merck, Germany. Nutrient agar media was used as culture media for enumeration of total bacteria in samples and incubated at 37°c for 18-24 hours. Membrane fecal coliform (mFC) agar media was used for the enumeration of total and fecal coliforms and petri-dishes were incubated at 37°C for 18 to 24 hours in the case of total coliform and in the case of fecal coliform at 44 to 44.5°C for overnight after inoculation following the methods of American Public Health Association (APHA) [5]. TCBS (Thiosulfate citrate bile sucrose) plate was appropriate selective media for identification of *Vibrio* spp. according to the Pfeffer and Oliver [6] and after 18-24 hours of incubation of TCBS plate at 37°C, slightly flattened, yellow with opaque centers colonies were considered as *Vibrio* spp. *Salmonella* spp. were counted on SS agar (*Salmonella-Shigella* agar) plate according to the Chowdhuri *et al.* [7] and colorless, transparent, with a black center colonies were considered as *Salmonella* after 18-24 h of incubation. Yeasts and molds spp. were identified and counted on OGYEA (Oxytetracycline-Glucose-Yeast Extract Agar) plates which were incubated at 22°C and examined for growth up to 5 days of incubation. OGYEA (Oxytetracycline-Glucose-Yeast Extract Agar) is superior for enumeration of yeast and molds spp. [8].

Statistical Analysis: Bacterial density data were transformed into natural log before statistical analysis. The means of bacterial load were compared using ANOVA followed by Tukey's post hoc for multiple comparisons. Statistical software Microsoft Excel, SPSS version 11.5 was used to analyze the data with the level of significance at $p<0.05$.

RESULTS

Comparison of Bacterial Density (CFU/g) of fresh *Nappi* Between Two Markets: The densities of TBC, FC, *Salmonella* and *Vibrio* spp. were significantly different ($p<0.05$) while the density of TC was more or less similar (Table 2). Between two markets, the higher densities of TBC ($1.43\pm0.01\times10^{10}$ CFU/g), TC ($7.75\pm0.19\times10^{9}$ CFU/g), FC ($5.87\pm0.11\times10^{9}$ CFU/g) and *Salmonella* spp. ($2.6\pm0.10\times10^{7}$ CFU/g) were found in Harbang and the higher density of *Vibrio* spp. ($3.35\pm0.25\times10^{6}$ CFU/g) was observed in Chaufaldandi.

Comparison of Bacterial Density (CFU/g) of stored *Nappi* Between Two Markets: The densities of TBC, FC, *Salmonella* and *Vibrio* spp. were significantly different

Table 2: Bacterial density (CFU/g) measured in fresh *Nappi* collected from Chaufaldandi and Harbang markets

Market Name	TBC	TC	FC	*Salmonella* spp.	*Vibrio* spp.
Chaufaldandi	$1.32\pm0.02\times10^{10b}$	$7.70\pm0.20\times10^{9}$	$5.06\pm0.01\times10^{9b}$	$2.30\pm0.1\times10^{7b}$	$3.35\pm0.25\times10^{6a}$
Harbang	$1.43\pm0.01\times10^{10a}$	$7.75\pm0.19\times10^{9}$	$5.87\pm0.11\times10^{9a}$	$2.6\pm0.10\times10^{7a}$	$3.25\pm0.05\times10^{6b}$

Means (± SEM) of 10 fresh *Nappi* samples within column with different letters (a and b) are significantly different (ANOVA, HSD; $p<0.05$)

TBC = Total bacterial count

TC = Total coliform count

FC = Fecal coliform count.

Table 3: Bacterial density (CFU/g) measured in stored *Nappi* collected from Chaufaldandi and Harbang markets

Market Name	TBC	TC	FC	*Salmonella* spp.	*Vibrio* spp.
Chaufaldandi	$1.63\pm0.02\times10^{10a}$	$8.55\pm0.15\times10^{9}$	$6.99\pm0.13\times10^{9a}$	$9.5\pm0.30\times10^{7a}$	$5.55\pm0.35\times10^{6a}$
Harbang	$1.52\pm0.01\times10^{10b}$	$8.25\pm0.05\times10^{9}$	$6.07\pm0.14\times10^{9b}$	$3.01\pm0.45\times10^{7b}$	$4.0\pm0.3\times10^{6b}$

Means (± SEM) of 10 stored *Nappi* samples within column with different letters (a and b) are significantly different (ANOVA, HSD; $p<0.05$)

TBC = Total bacterial count

TC = Total coliform count

FC = Fecal coliform count.

Table 4: Bacterial density (CFU/g) measured in fresh and stored *Nappi* collected from Chaufaldandi market

Phases of *Nappi*	TBC	TC	FC	*Salmonella* spp.	*Vibrio* spp.
Fresh	$1.32\pm0.02\times10^{10b}$	$7.70\pm0.20\times10^{9}$	$5.06\pm0.01\times10^{9b}$	$2.30\pm0.1\times10^{7b}$	$3.35\pm0.25\times10^{6b}$
Stored	$1.63\pm.02\times10^{10a}$	$8.55\pm0.15\times10^{9}$	$6.99\pm0.13\times10^{9a}$	$9.5\pm0.30\times10^{7a}$	$5.55\pm0.35\times10^{6a}$

Means (± SEM) of 10 fresh and stored *Nappi* samples within column with different letters (a and b) are significantly different (ANOVA, HSD; $p<0.05$)

TBC = Total bacterial count

TC = Total coliform count

FC= Fecal coliform count.

Table 5: Bacterial density (CFU/g) measured in fresh and stored *Nappi* collected from Harbang markets

Phases of *Nappi*	TBC	TC	FC	*Salmonella* spp.	*Vibrio* spp.
Fresh	$1.43\pm0.01\times10^{10b}$	$7.75\pm0.19\times10^{9}$	$5.87\pm0.11\times10^{9b}$	$2.6\pm0.10\times10^{7b}$	$3.25\pm0.05\times10^{6b}$
Stored	$1.52\pm0.01\times10^{10a}$	$8.25\pm0.05\times10^{9}$	$6.07\pm0.14\times10^{9a}$	$3.01\pm0.45\times10^{7a}$	$4.0\pm0.3\times10^{6a}$

Means (± SEM) of 10 fresh and stored *Nappi* samples within column with different letters (a and b) are significantly different (ANOVA, HSD; $p<0.05$)

TBC= Total bacterial count

TC= Total coliform count

FC = Fecal coliform count.

Table 6: Total density (CFU/g) of Yeast and Molds spp. in fresh and stored *Nappi* collected from two different markets (Chaufaldandi and Harbang)

Market Name	Yeast and Molds spp. (CFU/g)
Fresh (Chaufaldandi)	$4.7\pm0.1\times10^{6c}$
Stored (Chaufaldandi)	$5.70\pm0.13\times10^{6b}$
Fresh (Harbang)	$5.05\pm0.16\times10^{6b}$
Stored (Harbang)	$6.85\pm0.15\times10^{6a}$

Means (± SEM) of 10 fresh and stored *Nappi* samples within column with different letters (a, b and c) are significantly different (ANOVA, HSD; $p<0.05$)

($p<0.05$) while the density of TC was more or less similar (Table 3). The higher densities of TBC ($1.63\pm.02\times10^{10}$ CFU/g), TC ($8.55\pm0.15\times10^{9}$ CFU/g), FC ($6.99\pm0.13\times10^{9}$ CFU/g), *Salmonella* ($9.5\pm0.30\times10^{7}$ CFU/g) and *Vibrio* spp. ($5.55\pm0.35\times10^{6}$ CFU/g) were found in Chaufaldandi.

Comparison of Bacterial Density (CFU/g) Between Fresh and Stored *Nappi* in Chaufaldandi: The densities of TBC, FC, *Salmonella* and *Vibrio* spp. were significantly different ($p<0.05$) while the density of TC was more or less similar (Table 4). The densities of TBC ($1.63\pm.02\times10^{10}$ CFU/g), TC ($8.55\pm0.15\times10^{9}$ CFU/g), FC ($6.99\pm0.13\times10^{9}$ CFU/g), *Salmonella* ($9.5\pm0.30\times10^{7}$ CFU/g) and *Vibrio* spp. ($5.55\pm0.35\times10^{6}$ CFU/g) were found higher in stored than fresh *Nappi*.

Comparison of Bacterial Density (CFU/g) Between Fresh and Stored *Nappi* in Harbang: The densities of TBC, FC, *Salmonella* and *Vibrio* spp. were significantly different ($p<0.05$) while the density of TC was more or less similar (Table 5). The densities of TBC ($1.52\pm0.01\times10^{10}$ CFU/g),

TC ($8.25\pm0.05\times10^9$ CFU/g), FC ($6.07\pm0.14\times10^9$ CFU/g), *Salmonella* ($3.01\pm0.45\times10^7$ CFU/g) and *Vibrio* spp. ($4.0\pm0.3\times10^6$ CFU/g) were found greater in stored than fresh *Nappi*.

Comparison between Yeast and Molds spp. density (CFU/g) in *Nappi*: The density of Yeast and Molds spp. in *Nappi* were found significantly different ($p<0.05$) (Table 6). In Harbang both fresh and stored *Nappi* have the higher density of Yeast and Molds spp. ranging $5.05\pm0.16\times10^6$CFU/g $6.85\pm0.15\times10^6$CFU/g respectively than the other one. Between fresh and stored *Nappi* from both Chaufaldandi and Harbang, the higher density of Yeast and Molds spp. was found in the stored ($5.70\pm0.13\times10^6$ and $6.85\pm0.15\times10^6$CFU/g respectively).

DISCUSSION

Near about 45% ethnic people of Bangladesh consume *Nappi*. Fermented fishery products are not uniformly consumed over Bangladesh but it is popular among the ethnic people of Patuakhali, Barguna, Cox's Bazar, Chittagong and Chittagong hill tracts. It is also very popular foodstuff in Myanmar, Philippine, Indonesia, Thailand, China, Honkong and Malaysia [2].

Nappi production practices and marketing system are explicitly controlled by the ethnic people lacking proper knowledge of sanitation and hygiene. That makes the avenue of contamination and spoilage by microorganisms in different stages of production and marketing.

This study revealed that higher density of TBC (total bacterial count) was found in Harbang ($1.43\pm0.01\times10^{10}$ CFU/g) than Chaufaldandi ($1.32\pm0.02\times10^{10}$ CFU/g) for fresh *Nappi* category (Table 2). The reason of this high number of bacteria may be due to using contaminated raw products i.e. shrimps and/or processing in unhygienic condition. Naturally, fish carry a high number of bacteria due to their surrounding aquatic environment. The contamination of raw product was initiated with the improper post-harvest activities.

We found that the density of TBC (total bacterial count) in Chaufaldandi ($1.63\pm.02\times10^{10}$ CFU/g) is higher than Harbang ($1.52\pm0.01\times10^{10}$ CFU/g) for stored *Nappi* (Table 3). This may be due to the storage of *Nappi* of Chaufaldandi in more unhygienic condition than Harbang market. Nyarko *et al.* [9] found that the total bacterial load of smoked sardine *(Sardinella aurita)* in smoking sites ranged from 6.2×10^4 to 3.3×10^5 CFU/g while 7.2×10^4 to 4.1×10^7 CFU/g in marketing centres. Oku and Amakoromo [10] found that the total bacterial counts for fresh fish samples ranged from 4.0×10^8 - 2.30×10^{10} CFU/g and 1.8×10^4 - 2.5×10^8 CFU/g for smoked fish. Ibrahim *et al.* [11] found that the bacterial load for the fresh fish was 1.84×10^6 CFU/ml. and for the smoked fish 2.06×10^6 CFU/ml. Kakati and Goswami [12] found that the bacterial load in fermented fish product *Shidol* prepared from *Puntius sophore* was 5.4 log CFU/g and 5.1 log CFU/g for *Setipinna phasa*. Abolagba and Igbinevbo [13] found that the total bacterial count for fresh Clarias gariepinus was 1.35×10^5CFU/g and 450×10^5CFU/g for smoked *Clarias gariepinus*.

In the case of fresh *Nappi*, the higher densities of TC (total coliform) and FC (fecal coliform) were found in Harbang market ($7.75\pm0.19\times10^9$ and $5.87\pm0.11\times10^9$ CFU/g respectively) than Chaufaldandi ($7.70\pm0.20\times10^9$ and $5.06\pm0.01\times10^9$ CFU/g respectively) (Table 2). Coliform bacteria are normally present in warm-blooded animal's feces. Detection of this huge number of coliform bacteria in this study indicates that the raw fishes which might be collected from an unhygienic condition or the usage of polluted water during processing. Therefore, fecal coliforms which may be produced by animal or human feces are considered more accurate indicator of contamination of food than the total coliforms. Harbang market was more contaminated with animal or human feces than Chaufaldandi. The higher densities of TC (total coliform) and FC (fecal coliform) were found in Chaufaldandi ($8.55\pm0.15\times10^9$ and $6.99\pm0.13\times10^9$ CFU/g respectively) than Harbang ($8.25\pm0.05\times10^9$ and $6.07\pm0.14\times10^9$ CFU/g respectively) for stored category (Table 3). This may be due to the poor storage facilities of *Nappi* in Chaufaldandi. Nyarko *et al.* [9] found that the coliform bacteria of smoked sardine *(Sardinella aurita)* in smoking sites ranged from 0.0 to 2.1×10^4 CFU/g while 4.7 to 2.0×10^2 CFU/g in marketing centers. Majumdar *et al.* [14] found that the total and fecal coliform counts in different species of marine fish samples ranged from $2.18\pm1.49\text{x}10^5$to$4.18\pm4.01\text{x}10^6$CFU/gand $1.48\pm1.47\text{x}10^4$to $2.54\pm1.95\text{x}10^5$ CFU/g. The higher number of coliforms and fecal coliforms found in the study indicates that the culture environment of source fishes or processing environment of the final product might be somehow contaminated by human or other warm blooded animal's excreta.

This study discloses that the higher density of *Salmonella* spp. was found in Harbang ($2.6\pm0.10\times10^7$ CFU/g) than Chaufaldandi ($2.30\pm0.1\times10^7$ CFU/g) and *Vibrio* spp. in Chaufaldandi ($3.35\pm0.25\times10^6$ CFU/g) than Harbang ($3.25\pm0.05\times10^6$ CFU/g respectively) for fresh *Nappi* samples (Table 2). In the case of stored *Nappi*, the higher densities of *Salmonella* and *Vibrio* spp. were

found in Chaufaldandi ($9.5\pm0.30\times10^7$ and $5.55\pm0.35\times10^6$ CFU/g respectively) than Harbang ($3.01\pm0.45\times10^7$ and $4.0\pm0.3\times10^6$ CFU/g respectively) (Table 3). This might be due to the contamination of raw product i.e. shrimps. *Salmonella* and *Vibrio* in aquaculture products mainly originates from the culture environment of shrimps and processing environment of *Nappi*. Fishes could be infected by *V. cholerae* either due to sewage contamination of water or by ingestion of aquatic vegetation and zooplankton infested with *V. cholerae* [15]. Several studies have shown that zooplankton including copepods [16], as well as other aquatic crustaceans i.e., crabs, shrimps, prawns, lobsters and blue green algae [17,18] are important reservoirs of *V. cholerae*. But sometimes, incidence of those bacteria in fish, shrimp or similar foods of aquatic habitats may be happened due to external contamination. Although species level determination of the microbes was out of scope in this study, but this high quantity of colonies of *Vibrio* and *Salmonella* increase the probability of having pathogenic *V. cholerae, S. typhi* or *S. paratyphi* in *Nappi* which may cause dangerous diseases such as diarrhea, typhoid, paratyphoid etc.

In both categories the higher density of yeast and molds spp. were found in Harbang ($5.05\pm0.16\times10^6$ and $6.85\pm0.15\times10^6$ CFU/g respectively) than Chaufaldandi ($4.7\pm0.1\times10^6$and $5.70\pm0.13\times10^6$CFU/g respectively) (Table 6). Yeast and molds spp. require low pH and arid conditions to flourish. Thus a pH value of 5.84 and a water activity of 0.67 are adequate to support the growth of yeast and molds, hence the high counts [19]. Begum *et al.* [20] found that the total yeast counts of five freshwater fish samples in local markets ranged from 3.1×10^2 to 2.22×10^3 CFU/g while 2.4×10^2 to 1.78×10^3 in super shops. And total molds counts of five freshwater fish samples in local market ranged from 0.00 to 8.0×10^2 CFU/g while 0.00 to 6.3×10^2 CFU/g in super shops. Oku and Amakoromo [10] found that the fungal counts for fresh fish samples ranged from 1.8×10^4 - 7.0×10^4 CFU/g and 1.0×10^4 - 4.0×10^5 CFU/g for smoked fish. Nyarko *et al.* [9] found that the load of yeast and molds of smoked sardine *(Sardinella aurita)* in smoking sites ranged from 1.1×10^2 to 9.3×10^4CFU/g while 5.0×10^2 to 8.0×10^4 CFU/g in marketing centres. Abolagba and Igbinevbo [13] found that the fungal counts for fresh *Clarias gariepinus* was 15.7×10^5CFU/g and 300×10^5CFU/g for smoked *Clarias gariepinus*.

Between fresh and stored *Nappi* from both markets, the higher densities of TBC ($1.63\pm.02\times10^{10}$ and $1.52\pm0.01\times10^{10}$CFU/g respectively), TC ($8.55\pm0.15\times10^9$and $8.25\pm0.05\times10^9$CFU/g respectively), FC ($6.99\pm0.13\times10^9$and $6.07\pm0.14\times10^9$ CFU/g respectively), *Salmonella* ($9.5\pm0.30\times10^7$ and $3.01\pm0.45\times10^7$ CFU/g respectively), *Vibrio* spp. ($5.55\pm0.35\times10^6$ and $4.0\pm0.3\times10^6$ CFU/g respectively) and Yeast and Molds spp. ($5.70\pm0.13\times10^6$ and $6.85\pm0.15\times10^6$ CFU/g respectively) were found in stored samples (Table 4 and 5). This could be due to keeping the stored *Nappi* in an open air for one month which might increase the possibilities of huge bacterial growth.

This high number of pathogenic species such as *Salmonella* and *Vibrio* spp. may cause serious illness in ethnic community but the incidence is not common as because the ethnic people traditionally consume *Nappi* after cooking (more than 100 degree Celsius) with other ingredients. At this high temperature nearly all vegetative cells are died. According to Talaro and Chess [21], boiling water for 30 minutes will kill most non-spore-forming pathogens, including resistant species such as the tubercle bacillus and staphylococci etc. Ali *et al.* [22] found that the mean total coliforms in Cooked IQF shrimp was <3 ±0.00 MPN/g, while it was 23.50 ± 13.72 MPN/g in raw block frozen shrimp. Hossain *et al.* [23] also found that the bacterial density of the Cooked IQF shrimp was lower than Raw Block Frozen shrimp and Raw IQF shrimp and suggested that Cooked IQF shrimp was much better than other shrimps from the microbiological point of view. Again, continuous exposure to low dose of pathogen increases the disease resistant power [24, 25].

Nappi is a traditional delicacy, which is consumed by ethnic tribal community in their daily meal. The ethnic community may be suffered by many serious diseases for these high quantities of TBC, TC, FC, *Salmonella*, *Vibrio* and Yeast and Molds spp. when they consume *Nappi* without proper processing.

CONCLUSIONS

It was concluded that *Nappi* collected from the Chaufaldandi and Harbang markets was heavily contaminated with bacteria and yeast and mold spp. The contamination of *Nappi* mainly occurs by using spoiled raw shrimps and not following hygienic and sanitary processing and marketing techniques. Mainly *Nappi* is produced by poor ethnic community of Bangladesh especially Rakhaing who earn their income selling it. If sanitary and hygienic techniques are followed in the whole process of *Nappi* production; then it might improve the quality of *Nappi.* Improved product can take the potential international market of *Nappi* in Myanmar, Philippine, Indonesia, Thailand, China, Honkong and Malaysia.

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