

Comparative microbiological analysis of four different sea fishes collected from local market in Dhaka Metropolis

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Abstract

The present investigation attempted to evaluate the existence and survival of spoilage microorganisms in four common sea fishes (Poma, Rupchanda, Koral and Tuna) available in Bangladesh and to determine the effects of cooking temperature to optimize the growth of fish microflora. Moreover, the status of fish in frozen condition after cooking was also studied. A total 4 categories of sea fishes were collected from the local shops in Dhaka city. Raw, cooked and frozen fish samples were analyzed for the existence of pathogenic bacteria through the conventional cultural techniques and the confirmative biochemical identification procedures. Total viable bacteria were present in all four fish samples in raw, cooked and frozen condition up to 6 log CFU/mL. Most of the raw fish samples were found to harbor a huge population of microorganisms up to 5 log CFU/mL including the fecal coliforms. Several specific bacterial species like *E. coli*, *Klebsiella* spp., *Salmonella* spp., *Shigella* spp., *Staphylococcus* spp., *Pseudomonas* spp. and *Vibrio* spp. were present in raw samples. However, the microbial load reduced from the fish after cooking and the status was static in frozen condition. Thus, the incidence of fecal coliforms in raw fish may be considered as a serious threat to the public health upon consumption of such fishes.

1. Introduction

Fish and fish products are important source of animal protein, highly unsaturated fatty acid (HUFA) and polyunsaturated fatty acid (PUFA), minerals and vitamin. Marine fish oil is a good source of omega-3 fatty acids (Huynh *et al.*, 2007; Dhaneesh *et al.*, 2012; Belton *et al.*, 2014). In Bangladesh, about 60% of the total animal protein intake is obtained from fishery products (Bogarda *et al.*, 2015; Eizenberga *et al.*, 2015). Marine water of Bangladesh also has 442 species of fish and 36 species of marine shrimps and which is widely consumed by Bangladeshi people and tourists (Quader, 2010).

Because of its high nutritive value, sea fishes are a major vehicle for pathogenic bacteria. Fishes can be contaminated by both aquatic environment and post-harvesting condition (Al-Sheraa, 2018). Due to the attack of pathogenic bacteria and fungi a wide ranges of sea fishes spoilages occurs which adversely affect the economic condition in Bangladesh and as well as public health. Contamination of sea fishes can take places at several stages of transport, handling, processing,

packaging and storage condition by both bacteria and fungi. It was reported that Processing materials, water and ice could be a source of contamination (Sanjee and Karim, 2016). However, normal flora of fish proceeds spoilage due to inappropriate packaging (Bryan, 1980; Okonko *et al.*, 2008; Okonko *et al.*, 2009). *Aeromonas*, *Vibrio* spp., *Pseudomonas* spp., *Staphylococcus* spp., *Salmonella* spp., *Listeria* spp., *Clostridium perfringens*. are mainly caused various foodborne illness when they enter our intestine through contaminated sea fishes (Feldhusen, 2000; Vazquez-sanchez *et al.*, 2012; Zarei *et al.*, 2012; Falaise *et al.*, 2016; Iwamoto *et al.*, 2000). Along with bacteria and fungi, seafood-associated illness occurring by viruses (Norovirus and Hepatitis A) and certain parasites. Most outbreaks of food poisoning associated with fish and seafood derive from the consumption of raw or insufficiently heat treatment, insufficient cooking and cross-contamination during processing (Mohammed *et al.*, 2017).

Raw fishes naturally have a number of bacteria, and this can be opportunistic and causing foodborne infections rapidly if it is left for several hours at room temperature without processing. Pathogenic bacteria

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especially *Salmonella* spp. and *Vibrio* spp. are the primary concern of food safety with regard to seafood. When seafood is processed with uncontaminated water and cooked properly, it lowers the tendency the risk of food poisoning. Lack of proper temperature control is significant factors that can lead to pathogen growth and foodborne illness. Frozen fish are prone to contaminated by *Listeria* spp. (Reij et al., 2004; Jelena et al., 2011). Previously several studies showed that some thermostable enterotoxin of *Staphylococcus aureus* and *Escherichia coli* would not destroy completely even after cooking of seafood or if the food cooked properly, due to improper handling recontamination can take place and consumption of this type of food causes severe food poisoning (Ayulo et al., 1994; Vieira et al., 2001; Omeo et al., 2005; Simon and Sanjeev, 2007; Beleneva, 2011; Tango et al., 2015). On the other hand, cooked-frozen fish are widely consumed by working people in their busy life. They cooked fish and kept it in the refrigerator for 3-7 days or more. During this period the shelf-life of fish its organoleptic and chemical properties can be changed. Presence of *S. aureus* and *Bacillus cereus* was recorded in some cooked-frozen fish and meat as they are able to grow at refrigeration temperatures though a lower growth rate (Nyati, 2000; Rybka et al., 2011; Shakila et al., 2011). Along this line, it was clear that microbes can be found in any of the three conditions. So, the aim of our present studies is to determine the comparative microbiological quality among raw, cooked and cooked-frozen fish.

2. Materials and methods

2.1 Study area and sample collection

For the analysis of microbial load, Four fish samples (Rupchada, Koral, Tuna, Poma) were collected from the local market in Dhaka city using a sterile aseptic container together with ice. A total of 20 g of raw, cooked and cooked frozen sample of each fish was homogenized with 180 g of sterile normal saline. The homogenized suspension was subjected to serial dilutions (10-fold) up to 10^6 with normal saline (APHA 1998).

2.2 Enumeration of total viable bacteria and fungus

For enumerating total viable bacteria (TVB) and total fungal count, 0.1 mL of each sample was spread onto Nutrient agar (NA) and Sabouraud dextrose agar (SDA) respectively. For TVB, plates were incubated at 37°C. For fungal assay, plates were incubated at 25°C for 3 days (Sharmin et al., 2014).

2.3 Isolation of total coliform and fecal coliform

For the isolation of coliforms and fecal 0.1 mL suspension was spread over MacConkey agar and mFC agar. For the isolation of *E. coli* and *Klebsiella* spp., plates were incubated at 37°C for 18-24 hrs. The presence of *E. coli* was further confirmed by the appearance of bluish-black colonies with green metallic sheen on eosin-methylene blue (EMB) agar. While for fecal coliforms, plates were incubated at 44.5°C for 24 hrs (Sharmin et al., 2014).

2.4 Assay of pathogenic bacterial load

For the isolation of *Salmonella* spp., *Shigella* spp., *Vibrio* spp. and *Staphylococcus* spp., 0.1 mL of suspension was spread onto Xylose Lysine Deoxycholate (XLD), Thiosulphate Citrate Bile Salt Sucrose (TCBS) agar plates and Mannitol salt agar (MSA) respectively. After incubation at 37°C for 24 hrs, characteristic colonies were enumerated (Sharmin et al., 2014).

3. Results and discussion

The spoilage of sea fish caused by different bacteria is not very infrequent. Therefore, it is very essential to ensure the quality of fish as well as the consumer's safety. In our previous study, we were able to identify a huge array of microbial growth in different sea fish samples of which most were drug-resistant (Noor et al., 2013). In the current study, higher pathogenic loads were found in all the category of four sea fish samples employed and all the isolates were biochemically identified (Table 1). The bacterial contamination was very high in raw fish samples rather than the cooked and frozen fish. The total viable bacteria and fungal growth were found in raw fish poma fish nearly 7 log CFU/mL. Total viable bacteria were also present in cooked and frozen poma fish up to 6 log and 4.5 log CFU/mL respectively. In case of specific pathogens like *E. coli*, *Klebsiella*, *Staphylococcus* spp., *Shigella* spp., *Salmonella* spp., *Pseudomonas* spp. and *Vibrio* spp. were present within the range of 2.5 - 4.0 log CFU/mL. Frozen poma fish was totally contamination-free in case of all pathogens but *E. coli* and *Pseudomonas* spp. was present in cooked poma fish. Both raw and cooked poma fish were found to be fecally contaminated (Figure 1A). In the case of rupchanda fish, raw, cooked and frozen state was found to be contaminated by viable bacteria up to 5.5 log CFU/mL. The specific pathogens were noticed in raw rupchanda fish up to 5 log CFU/mL whereas *E. coli* and *Staphylococcus* spp. were present in frozen rupchanda within the range of 2.2 - 3.5 log CFU/mL. The growth of *Shigella* spp. was absent in all 3 categories of rupchanda fish. Raw rupchanda fish showed the existence of fecal bacteria (Figure 1B).

Table 1. Biochemical identification of the bacterial isolates from sea fish.

| Assumed Pathogenic Microorganisms | TSI | | | | Motility | Indole Production | MR | VP | Citrate utilization | Catalase | Oxidase |
|-----------------------------------|-------|------|-----|------------------|----------|-------------------|----|----|---------------------|----------|---------|
| | Slant | Butt | Gas | H ₂ S | | | | | | | |
| <i>E. coli</i> | Y | Y | + | - | + | + | + | - | - | + | - |
| <i>Klebsiella</i> spp. | Y | Y | + | - | + | - | - | + | + | + | - |
| <i>Vibrio</i> spp. | R | Y | - | - | + | - | + | - | - | + | + |
| <i>Staphylococcus</i> spp. | Y | Y | - | - | + | - | + | - | - | + | - |
| <i>Pseudomonas</i> spp. | R | Y | - | - | + | - | + | - | - | + | + |
| <i>Salmonella</i> spp. | R | Y | - | - | + | - | + | - | - | - | - |
| <i>Shigella</i> spp. | R | Y | + | - | - | +/- | + | - | - | - | - |

All the experiments have been done three times and the results were reproducible. One representative data have been shown. TSI: Triple Sugar Iron Test; Y: Yellow (Acid); R: Red (Alkaline); MR: Methyl red and; VP: Voges-Proskauer

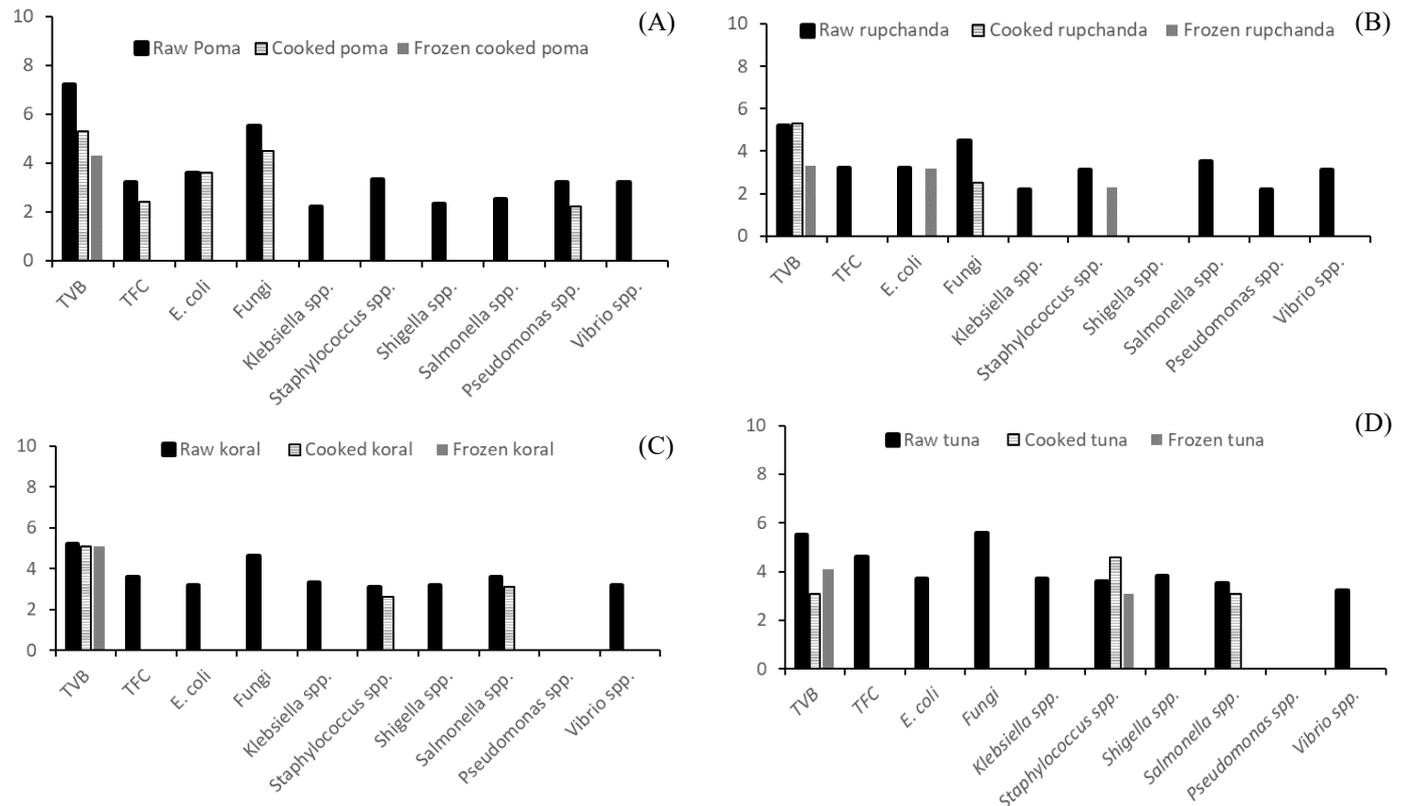


Figure 1. Microbiological status of different sea fish: poma fish (A); rupchanda fish (B); koral fish (C); and tuna fish (D).

Meanwhile, the raw koral fish exhibited the huge array of *E. coli*, *Klebsiella* spp., *Staphylococcus* spp., *Shigella* spp., *Salmonella* spp., *Pseudomonas* spp. and *Vibrio* spp. up to 4 log CFU/mL. Only *Staphylococcus* spp. and *Salmonella* spp. were present in cooked koral samples up to 3.7 log CFU/mL. All three categories exhibited total viable bacteria. Only raw koral fish had fungal load (Figure 1C).

The raw tuna fish showed huge fungal contamination as well as total viable bacteria which was recorded within the range of 5.5 to 6 log CFU/mL (Figure 1D). In case of specific bacteria, *E. coli*, *Staphylococcus* spp., *Shigella* spp., *Salmonella* spp. and *Vibrio* spp. was present in raw tuna fish up to 4.5 log CFU/mL while *Pseudomonas* spp. was absent in raw, cooked and frozen tuna. *Staphylococcus* spp. was present in raw, cooked and frozen samples within the range of 2.7 log to 4.3 log

CFU/mL (Figure 1D). Only the raw tuna showed fecal contamination.

As described in our early study, several factors may affect the overall quality of the food and fish such as contaminated ice, transportation and poor storage condition or due to cross-contamination from other fish (Noor et al., 2013).

4. Conclusion

The results of the current study confirmed the existence of contaminating microorganisms in sea fish especially in raw samples. In the current study, the cooked and frozen samples were quite satisfactory rather than the raw samples. This study tried to sort out the cooking effects on the reduction of existence microorganism in the fish samples as well as the various

consequences may happen during the frozen condition. The bacterial load was remarkably reduced after cooking and even the quality was sustained in the frozen state. In the future, it would be important to evaluate the drug resistance traits of the fish microflora to ensure the consumer's health. However, the major outcome of this very study is the cooking effects in proper temperature may reduce the microbial spoilage in food and fish.

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