

Assessment of sensory and microbiological quality of five marketed fish species at Dhaka city in Bangladesh

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Abstract

The study assessed the sensory quality and the bacterial condition in five nutritionally important fish species at Dhaka city in Bangladesh. Sensory defect points (DP) were analysed at *in-situ* conditions and microbiological attributes were conducted at laboratory condition. Sensory DP indicated fishes in retail markets (RM) were deteriorating and/or spoiled (DP >3 to <5) except live fishes. In super shops (SS), DP indicated excellent to good quality (DP >1 to <3) for all the fishes. The average total plate count (TPC) in the fishes from RM exceeds the permissible limit (PL) except for the fishes sold in live condition. A highly significant difference observed in the fishes sold in dead condition between RM and SS ($p \leq 0.001$). Correlation analysis revealed an insignificant relationship between sensory DP and TPC ($p \geq 0.05$) in the markets. The study recorded total coliform counts (TCC) was within the PL i.e., <100 MPN/g in both types of markets. Faecal coliform counts (FCC) were within the acceptable limit i.e., <10 MPN/g only in the fishes from super shops. *Salmonella* spp. and *Vibrio cholerae* was detected almost in all samples from retail markets while it was very less likely detected in SS. The study revealed that fish from both RM and SS is safe for human consumption but super shops possess better quality than retail markets.

1. Introduction

Fish is a vital source of food as well as a dominant source of high-quality protein in the human diet (Tidwell and Allan, 2001), particularly in the low- and middle-income countries in the global south (Kwasek *et al.*, 2020). However, fish has been considered as a widely implicated food that is frequently associated with human disease outbreaks (Novoslavskij *et al.*, 2015; Barrett *et al.* 2017). Hence, the determination of freshness quality of fish is becoming popular in modern research and development to ensure food safety (Dalgaard *et al.*, 2006; Cheng, 2015). A variety of the indices such as sensory attributes, microbial contamination and chemical changes have been used for the assessment of fish quality during harvesting, distribution and storage (Alam, 2007; Oehlenschlager, 2013; Cheng *et al.*, 2015). Sensory evaluation entails the understanding of the

acceptability of the fish based on organoleptic characteristics such as physical appearance, consistency of the flesh, odour, texture, eye, gill colour and more for a long time (Martinsdottir, 2002; Nowsad, 2010; Howgate, 2015). Several methods of sensory assessment in fish and fishery products have been described such as quality index method (QIM) and defect point (DP) analysis (Hyldig and Green-Petersen, 2005; Nowsad, 2010). In DP analysis, a score between 3 to 4 denotes fishes are deteriorating and not acceptable for human consumption, whereas 4 to 5 means fishes are spoiled (Connell, 1990). Further, DP score 3.3 as sensory quality breaking point which means at this point fish can be considered as rejected (Nowsad, 2010).

Microbial contamination is another major cause of fish quality deterioration that occurs from nature as well as during post-harvest processing and marketing (Ghaly

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et al., 2010; Svanevik et al., 2015; Ikape, 2017). Studies enumerate bacterial load i.e., total bacterial count (TBC) in fish is used to predict the freshness and degree of spoilage or contamination (Cheng et al., 2015; Sanjee and Karim, 2016). Coliform bacteria, both total and faecal, indicate the level of sewage contamination and pollution in the aquatic environment which may also occur during processing and retailing through handling, washing water and ice (Mandal et al., 2009; Sichewo et al., 2014; Sanjee and Karim, 2016; Ariyawansa et al., 2016). In fresh fish, an acceptable limit for TBC is $5.70 \log_{10}$ CFU/g whereas total and faecal coliform are <100 MPN/g and <10 MPN/g, respectively (ICMSF, 1986). Naturally occurring *Salmonella* spp. in fish is a leading cause of foodborne illness (Gnanambal and Patterson, 2005; Iwamoto et al., 2010). Moreover, fish act as a reservoir of *Vibrio cholerae* which causes epidemic cholera transmitted to human through ingestion (Senderovich et al., 2010; Halpern and Izhaki, 2017). In Bangladesh, consumers are used to buying fish from retail markets and super shops. In Dhaka city, a substantial percentage of fish come to retailers' shops through a long value chain from distant sources that do not follow proper handling and cool chain management practices (Apu, 2014; Nowsad, 2015). Generally, hygiene practices in fish retail markets are considered very poor (Alam et al., 2014). Moreover, retailers use contaminated water from nearby ponds or rivers for washing and provide a very small amount of ice for keeping the fishes cool. Furthermore, they keep the fish on unhygienic substances or floor at ambient temperature and use bare hands to weigh and package fish during transactions. These factors might increase the prevalence of bacterial multiplication in raw fish as reported in different food items including fish products (Svanevik et al., 2015; Visnuvinayagam et al., 2017; Yap et al., 2019).

On the contrary, super shops are becoming popular to consumers in Dhaka city of Bangladesh due to a neat and clean environment with attractive facilities. In general, super shops collect freshwater fishes from farmers, *haor* areas and wholesale markets in Dhaka and marine fishes from Chattogram (Rahman et al., 2017). The value chain is almost similar to retail markets and therefore always a risk of contamination through unhygienic handling and transportation. In Bangladesh, several studies conducted on sensory evaluation and microbiological analysis in fishes are sourced from the farm, wild (freshwater and marine water), landing centre, value chain nodes and retail markets. However, there is a strong lack of information on comparative analysis of sensory and microbiological assessment between fish from retail markets and super shops. A better

understanding of the sensory and microbial attributes in fresh fish from the markets could be very crucial in controlling disease outbreaks caused by fish consumption, thus minimize health risks. Therefore, the main objectives of this study are to evaluate the sensory and microbiological quality of five marketed fish species collected from both retail markets and super shops which provide basic information about the quality of fishes and existing markets condition that would be helpful to consumers, policymakers and researchers.

2. Materials and methods

2.1 Sample collection

Five fish species namely mola (*Amblypharyngodon mola*), bata (*Labeo bata*), koi (*Anabas testudineus*), tengra (*Mystus tengara*) and jat puti (*Puntius sophore*) collected from three retail markets (RM) and three super shops (SS) in Dhaka city (Figure 1). A total of ten specimens of each fish from each station were randomly collected for sensory analysis and ten for microbiological assessment. Immediately after sample collection, a sensory analysis was carried out on the spot while the samples for microbial assessment were kept in an aseptic sterilized zipper plastic bag, store in an insulated icebox and finally transported to the laboratory.

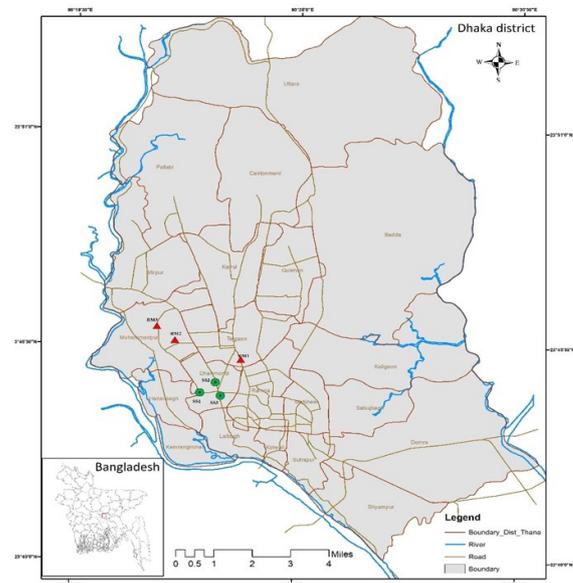


Figure 1. Sampling sites (red colour indicates retail markets and green colour indicates super shops)

2.2 Sensory quality assessment

The sensory quality of marketed fishes was measured following the sensory defects and defect points (DP) chart modified by Nowsad (2010). The method is rationally revised based on the Torry research station approach by Howgate et al. (1992). Finally, the acceptability of the raw fish was calculated from the grading category as in Table 1 (Nowsad, 2010).

Table 1. Quality grading of fish against defect points (DP)

Grade	DP	Grade characteristics
A	<2	Excellent, highly acceptable
B	2 to 3	Good, acceptable
C	>3 to <4	Deteriorating, not acceptable
D	4 to 5	Spoiled, rejected

2.3 Sample preparation for microbiological assessment

Collected samples were preserved at -18°C and subsequently, each species from individual stations was blended in a sterilized blender after washing with Butterfield' phosphate buffer solution (PBS). In an aseptic blender jar, 50 g of fish samples and 450 mL of PBS were mixed and subject to homogenization in a vortex machine. After proper homogenization, 1 mL sample was transferred into a sterile cap tube containing 9 mL autoclaved 0.1% peptone water to produce 10⁻¹ dilution. Then, 1 mL of the original dilution (10⁻¹) was transferred to prepare further decimal dilutions up to 10⁻³.

2.4 Enumeration of total plate counts (TPC)

Bacteriological peptone plate count agar (Difco™ plate count agar) was used for the enumeration of bacteria from the collected fish samples. The spread plate method described by ISO (2003) was used for inoculation and all the inoculated Petri-dishes were incubated for 18-24 hrs at 37°C. Finally, colony-forming units (CFU/mL) were counted considering the plates having well-spaced colonies (30 to 300). Finally, the total plate count was converted to and expressed as log₁₀ CFU/g. The total number of bacteria per mL of sample was obtained as follows:

$$\text{CFU /mL} = \frac{\text{(Number of colonies} \times \text{dilution factor)}}{\text{Volume of the culture plate}}$$

2.5 Total coliform counts (TCC)

TCC was enumerated according to ISO 4831:1991. For enumeration of total coliforms, Laurylsulfate-tryptose Broth (LSTB) was prepared and transferred into tubes and autoclaved after adding Durham's tubes (Pyrex). From processed fish samples, 1 mL of each of the 10⁻¹, 10⁻² and 10⁻³ dilutions were transferred into the three separate tubes of LSTB containing Durham's tube. The tubes were incubated in an incubator at 37°C for 48 hrs. After incubation, the positive gas production tubes were recorded. For each set of positive LSTB tubes, one set of Brilliant Green Bile Broth (BGBB) tubes (for total coliform enumeration) and one set of EC tubes were prepared. A loopful of broth from each positive LSTB tubes of culture was inoculated into a tube of BGBB and incubated at 37°C for 24 hrs. The positive gas production tubes were recorded and the result was computed as MPN/g.

2.6 Faecal coliform counts (FCC)

FCC was enumerated according to ISO 7251:1993. To enumerate faecal coliforms a loopful broth from the tubes of LSTB that were positive for gas production were transferred to EC containing Durham's tubes. EC tubes were incubated at 45°C for 24 hrs in a circulatory water bath. After incubation, positive gas production tubes were recorded and from each tube, a loopful of broth was transferred to a sterile tryptone water tube. Tubes were incubated at 37°C for 48 hrs in an incubator and after incubation Kovac's reagent were added to determine the presence of indole ring. A positive indole reaction indicates the presence of *Escherichia coli*. Positive tubes were recorded and the results were computed using MPN/g chart.

2.7 Identification of *Salmonella* spp. and *Vibrio cholerae*

For the determination of *Salmonella* spp. and *Vibrio cholerae*, Salmonella Shigella (SS) agar and Thiosulfate Citrate Bile Salt Sucrose (TCBS) agar were used respectively. After incubation at 37°C for 18-24 hrs, colourless transparent and black centres colonies were considered as *Salmonella* spp. and yellow with opaque centres colonies were considered as *Vibrio cholerae*.

2.8 Statistical analysis

Pearson correlation analysis and Welch's t-test were carried at a 95% confidence interval (CI) to investigate the correlation between the sensory DP and TPC, and the statistical significance between RM and SS respectively.

3. Results and discussion

3.1 Sensory defect points of the fishes

Sensory defect points (DP) revealed that the average quality DP of mola and jat puti in retail markets (RM) ranges between >3 to 5 while sensory DP of koi and tengra were 1 to <3 (Table 2). Sensory DP of Bata in RM was within the good quality in RM2 and RM3 while in RM3 it was found above 3. In super shops (SS) average sensory DP for all species in all stations were <3. DP in koi and tengra DP were <2 which means excellent in quality. Bata also found with excellent quality in SS2 and SS3. The study revealed that fishes sold in dead condition were deteriorating in RM while in SS the same species remain in good quality. Only live or air-breathing fish were found between good to excellent quality in both type of markets. In retail markets in Dhaka city, Nowsad (2010) determined a more or less similar sensory DP of bata (2.9±0.2) sourced from Sylhet district but a higher DP in tengra (3.2±0.3) sourced from Jashore district. Ezung and Abraham (2013) measured quality index method (QIM) score in live bata 3.80 and tengra

Table 2. Sensory defect points (DP) fish samples

Markets	Fish species				
	Mola	Bata	Koi	Tengra	Jat puti
Retail markets					
LM1	3.88±0.46	3.02±0.34	1.12±0.09	2.02±0.37	3.02±0.15
LM2	4.08±0.47	2.77±0.31	1.13±0.10	2.30±0.38	3.75±0.50
LM3	3.20±0.28	2.81±0.26	1.27±0.24	2.05±0.28	3.40±0.55
Super shops					
SS1	2.11±0.48	2.02±0.35	1.03±0.05	1.30±0.19	2.55±0.37
SS2	2.40±0.49	1.82±0.23	1.03±0.08	1.44±0.18	2.05±0.31
SS3	2.63±0.37	1.98±0.39	1.04±0.06	1.54±0.30	2.35±0.38

5.40. QIM in Bata fish collected from Siliguri retail market in India was found 5 (Jha *et al.*, 2010). A low QIM indicates fishes were of good quality (Nielsen and Hyldig, 2004).

3.2 Total plate counts

Total plate counts (TPC) in all five fish species from the retail markets' and super shops in Dhaka city are presented in Table 3. Results revealed that TPC in the fishes collected from the retail markets is higher than the samples from the super shops. The highest average TPC was recorded in mola from RM ($6.54 \pm 0.06 \log_{10}$ CFU/g) while lowest in koi from SS ($5.11 \pm 0.04 \log_{10}$ CFU/g). The t-test revealed a significant difference in TPC in mola, bata and jat puti between retail markets and super shops at a 1% significance level ($p < 0.001$), in koi at a 10% significance level ($p < 0.05$). Tengra did not show any significant difference between RM and SS. TPC did not show a significant correlation ($P > 0.05$) with DP of the same species from the same market.

Table 3. Mean total plate counts (TPC) in fish samples

Fish species	TPC (\log_{10} CFU/g)			
	RM	SS	t-test (95%)	p-value
Mola	6.54±0.06	5.63±0.10	13.58	0.0007***
Bata	6.24±0.12	5.13±0.10	12.63	0.0003***
Koi	5.38±0.09	5.11±0.04	4.83	0.0231*
Tengra	5.44±0.10	5.34±0.06	1.58	0.2027
Jat puti	6.48±0.07	5.67±0.09	12.192	0.0004***

*, **, and *** indicate 10%, 5% and 1% significance level, respectively.

3.3 Coliform count

Total coliform counts (TCC) and faecal coliform counts (FCC) in the collected fish samples from RM and SS were shown similar trends like TPC, i.e. higher in RM and lower in SS (Figure 2). The highest average TCC was counted in jat puti from LM (97 ± 5 MPN/g) and lowest in bata (7 ± 4 MPN/g). On the other hand, the highest FCC was recorded in mola from RM (42 ± 6 MPN/g) and lowest in koi in SS (4 ± 1 MPN/g).

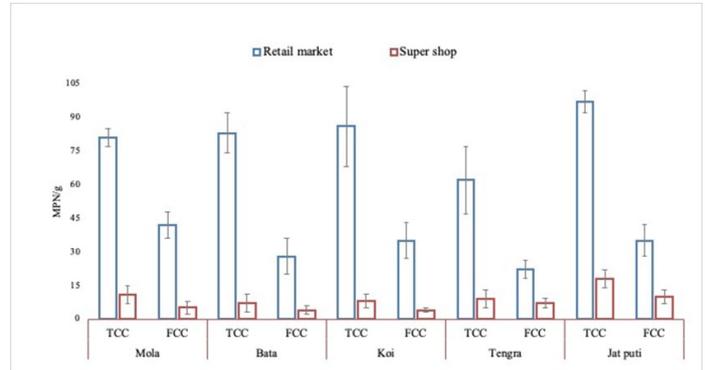


Figure 2. Total coliform counts (TCC) and faecal coliform counts (FCC) in fish samples

3.4 Occurrence of *Salmonella* spp. and *Vibrio cholerae*

In this study, the occurrence of *Salmonella* spp. and *Vibrio cholerae* were more frequent in retail markets than super shops (Table 4). *Salmonella* was detected in almost all fish samples except bata and jat puti in RM2 and, mola and bata in RM3. On the other hand, the presence of *Salmonella* spp. just only recorded in mola and koi in SS1, koi in SS2 and, mola and jat puti in SS3. Similarly, *Vibrio cholerae* was more abundant in retail markets than in super shops but less occurrence than *Salmonella* spp. This study found *Vibrio cholerae* in all samples from RM1 except mola while in super shops it was reported only in koi from SS2. A similar study on microbiological assessment in mola fish collected from the retail market and the super shops in Dhaka Metropolis recorded total bacterial count (TBC), total coliform count (TCC), total faecal coliform (TFC), *Salmonella* spp. and *Vibrio* spp. (Nilla *et al.*, 2012). The study found TBC range in retail markets between 5.08 to 6.63 \log_{10} CFU/g and in super shops 3.74 to 5.81 \log_{10} CFU/g. Moreover, the study reported a higher level of TCC, TFC, *Salmonella* spp. and *Vibrio* spp. in fish from retail markets than the super shops. TBC, TCC and pathogenic bacterial load in raw fish collected from different retail markets in Dhaka city exceeded the acceptable limit by ICMSF (Hasan *et al.*, 2012; Hasan *et al.*, 2015). High bacterial contamination in mola fish from retail markets in Savar, Dhaka was reported by Mahin *et al.* (2011) found total viable counts (TVC), TCC and TFC were 7.99 \log_{10} CFU/g, 5.80 \log_{10} CFU/g

Table 4. Occurrence of *Salmonella* spp. and *Vibrio cholerae* in fish samples

Markets	Fish species				
	Mola	Bata	Koi	Tengra	Jat puti
Retail markets					
LM1	S	S V	S V	S V	S V
LM2	S V	V	S	V	V
LM3	V		S V	S	S
Super shops					
SS1	S		S		
SS2			SV		
SS3	S				S

“S” indicates presence of *Salmonella* spp. and “V” indicates presence of *Vibrio cholerae*

and 4.99 log₁₀ CFU/g, respectively. Average TVC in bata and tengra were calculated as 5.71 log₁₀ Cfu/g and 6.22 log₁₀ CFU/g, respectively in retail markets in Kolkata, India (Ezung and Abraham, 2013). Similarly, a high microbial load such as TBC, TCC and TFC in raw fish from retail markets were reported in Siliguri, India (Jha *et al.*, 2010) and Lahore, Pakistan (Chatta *et al.*, 2018).

In retail markets, high microbial contamination in the fishes was reported due to no or delay in icing, rough handling, compactness, lack of sanitation, contaminated water, contaminated ice, soiled surfaces and boxes (Mhango *et al.*, 2010; Hasan *et al.*, 2015; Hossain and Barman, 2016). Further, fishes are left in ambient temperature in retail markets in Bangladesh with a small amount or no ice which greatly influence microbial activity (Alam, 2007). Eltholth *et al.* (2018) reported that lack of cool chain management during fish selling in retail markets increase the total bacterial load. We observed all the above scenarios in the retail markets whereas in super shops proper cooling and sanitation facilities were maintained. Moreover, a lower prevalence of pathogenic bacteria in super shops than in retail markets indicates better handling, cooling and sanitation maintenance during selling. Our study remarkably indicates that fish in SS is better than RM in terms of food safety since a higher occurrence of *Salmonella* spp. and *Vibrio cholerae* may cause foodborne infection to the consumers.

4. Conclusion

The study revealed that high microbial contamination, *Salmonella* spp. and *Vibrio cholerae* were frequently found in retail markets compared to super shops. This phenomenon is caused by poor icing, rough handling, lack of sanitation, contaminated water and ice, surfaces and containers. The ambient temperature, as well as unhygienic condition in retail markets, are responsible for increasing the total bacterial load, whereas in super shops cooling and sanitation facilities are maintained indicating better handling,

cooling and sanitation maintenance during selling. Consumers can be alerted about the contaminated state of the retail fish and can be urged to wash fish properly before cooking. Proper fish preservation and handwashing practice should be encouraged among vendors as well as fish handlers emphasizing the benefits of simple practices that promote safe health.

Conflict of interest

The authors declare no conflict of interest.

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