Nutritional, microbial and various quality aspects of common dried fish from commercial fish drying centers in Bangladesh

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\textbf{ABSTRACT}

The purpose of this study was to evaluate the nutritional, microbial and sensory quality of five dried fish species from five fish drying centers in Bangladesh, and consumers’ perception on quality and perceived health problems of consuming dried fish. Proximate composition and bacterial load were determined following AOAC and total plate count method, respectively. Data on peoples’ perception regarding the quality aspects of dried fish were collected using a structured questionnaire. Sensory analysis showed samples from Cox’s Bazar had higher acceptability than other regions. Moisture content ranged from 12.00 ± 1.12% to 22.99 ± 1.09%, the highest found in Bombay duck from Patuakhali. The highest values of protein were found in shrimp from Chittagong (64.33 ± 0.99%) and the lowest in Bombay duck from Bhola (51.60 ± 0.95%). The lipid content varied from 5.38 ± 0.37% (Bombay duck from Cox’s Bazar) to 8.67 ± 0.96% (Bombay duck from Khulna). The ash content was ranged from 13.89 ± 0.94% to 20.07 ± 1.64% in Bombay duck from Patuakhali and Cox’s Bazar. The mean total plate count of dried Bombay duck were 7.1 ± 0.2×10\textsuperscript{5}, 9.8 ± 0.1×10\textsuperscript{5} and 7.8 ± 0.52 ×10\textsuperscript{5} cfu/g, whereas, total Vibrio spp. count were estimated 1.1 ± 1×10\textsuperscript{3}, 3.7 ± 0.2×10\textsuperscript{3} and 1.8 ± 1×10\textsuperscript{2} cfu/g for Chittagong, Cox’s Bazar and Bhola, respectively. The pathogenic bacterial species \textit{E. coli} and \textit{Salmonella} were absent in dried Bombay duck from all locations. Of 500 respondents, the majority (94.8%) reported no complications after consuming dried fish. Significant quality variation among the dried fish samples suggested further improvement in dried fish quality through maintaining hygiene and sanitation to produce quality and safe dried fish for the consumers in home and abroad.

1. Introduction

Bangladesh’s fisheries sector contributed 3.57% and 25.30% to the national and agricultural gross domestic product (GDP), respectively, and earns approximately $5 million by exporting fish and fisheries products in 2017–18 (DoF, 2018). Additionally, by contributing 60% of the total animal protein, this sector helps to ensure food and nutrition security (DoF, 2018). Among all fisheries products, dried fish known as “Shutki” locally, is the cheapest source of essential amino acid containing protein along with essential fatty acids, several minerals and vitamins (Mazumder et al., 2008; Siddique and Aktar 2011).

Bangladesh is endowed with vast diversified fisheries resources (Hanif et al., 2015; Islam et al., 2017), and dried fish and fishery products are produced from a wide range of freshwater and marine water fish species, including Bombay duck, Chinese pomfret, Ribbon fish, Shrimp, and Silver jewfish (Paul et al., 2018). Moreover, several fish species such as Bombay duck and Ribbon fish are preferred to be taken as dried rather fresh condition (Hoque et al., 2021). Apart from that, people usually consumed dried fish because of its distinctive flavor and aroma (Hossain et al., 2013). Given this enormous demand, nowadays fish is dried commercially in several areas of Bangladesh. The major areas for dried fish production are Chittagong, Dublar Chor of Bagerhat, Moheshkhali, Sonadia and St. Martin Island of Cox’s Bazar, and Kuakata of Patuakhali (Hossain et al., 2013; Nowsad, 2007).

Despite potential market demand and the nutritional benefits of dried fish, there are a range of challenges with the traditional sun-drying process (Mithun et al., 2021; Reza et al. 2005; Roy et al. 2014). Dried fish may lose nutritional value if kept for an extended period of time...
because they absorb moisture from the environment (Sultana et al., 2010). Moreover, producers frequently use improper drying techniques to increase fish weight in an effort to make additional financial gains. During the storage period, producer(s) often use harmful insecticides to protect dry fishes from insect infestation and microbiological contamination, which could deteriorate the nutritional quality of dried fish and constitute a threat to public health (Reza et al., 2005; Mazumder et al., 2008; Sultana et al., 2010; Roy et al., 2014).

As traditionally processed food, quality of dried fish and fishery products is considered to be concern for public health. Consumers of Bangladesh are now more aware of health issues. However, the public health could be endangered by dried fish that has deteriorated nutritionally and is microbiologically contaminated. According to a study from Chittagong, Bangladesh, dry fish’s nutritional content decreases over time due to prolonged storage (Siddique and Aktar, 2011). Another study from Bangladesh found deteriorative changes in dried fish that could lead to browning reactions and the development of rancid and off-smelling odors when moisture content significantly higher (Mansur et al., 2013). Nutritional loss of dried fish may also result from spoiled raw materials, poor sanitation and personal hygienic conditions, conventional processing and long-term traditional storage conditions. In addition, microbial growth and activity is one of the major causes of fish spoilage and alteration in quality traits such as flavours, odours and colour. Dried fish have a chance to be contaminated by diseases causing and health hazardous microbial species like E. coli O 157:H7, and Salmonella sp. (Sultana et al., 2010; Rasul et al., 2020). The occurrence of Salmonella sp. in dried fish was a clear indication that poor sanitary practices were maintained during the fish drying process (Sultana et al., 2010).

There have been a few investigations on the dried fish quality aspects in Bangladesh, but all of which are limited to specific locations (such as local market of different regions) and fish species (Aktar et al., 2018; Mansur et al., 2013; Mazumder et al., 2008; Pravakar et al., 2013; Rasul et al., 2020; Siddique and Aktar, 2011). Hoque et al. (2021) studied the chemical contaminants (heavy metal and pesticides) in dried fish from coastal regions of Bangladesh. However, there hasn’t been any investigation done yet on the nutritional and microbial quality of mostly consumed dried fish collected from different commercial fish drying centers in Bangladesh. Unfortunately, often heard to complaint by the consumers about the quality of traditional sun-dried fishes available in

### Table 1. Grading of dried fish acceptance.

| Grade | Average Defect point | Degree of acceptance |
|-------|----------------------|----------------------|
| A     | < 2                  | Excellent, Highly acceptable |
| B     | 2 to < 5             | Good/acceptable       |
| C     | 5                    | Rejected              |

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local markets of Bangladesh (Reza et al., 2005). Since the dried fish available in local market comes from the commercial fish drying centers in coastal area of Bangladesh; thus, there is an emergent need to consistently monitor and evaluate the quality of dried fish produces in the commercial drying areas. The findings will explore the different quality aspects of dried fish which may raise the acceptability of dried fish to the consumers and also, able to create foreign market. Hence, the study aimed to evaluate the sensory, nutritional and microbial quality of common dried fish from different commercial fish drying centers in Bangladesh, as well as consumers’ perception on quality and perceived health problems of consuming dried fish.

2. Materials and methods

2.1. Study sites and period

The study was undertaken (survey data and sample collection) in five major fish drying centers of Patuakhali, Cox’s Bazar, Khulna, Chittagong, and Bhola in Bangladesh (Figure 1) from December 2018 to November 2019. The areas were purposively selected for this study. The areas are considered to be representative and suitable for the study as the leading points for landing and drying of marine fish from the Bay of Bengal, Bangladesh.

2.2. Study design and survey procedures

We designed a mixed-methods study, both community-based survey and laboratory-based analysis of samples, to assess the quality aspects (sensory, nutritional and microbial) of common dried fish as well as consumers’ perception on quality and perceived health problems of consuming dried fish in Bangladesh.

The survey was carried out among 500 (100 from each study area) Bangladeshi households in the mentioned vital areas (n = 5) for fish drying. Five trained data collectors, 2 from the research team and 3 recruited externally, visited the households situated near to the commercial fish drying centers and randomly recruited one adult (18 years age or older) from the selected households. The principal investigator of this study organized a training session to instruct the data collectors on sampling techniques, content of the questionnaire, data collection procedures and participants. For survey data collection, in-persons (face-to-face) interview were conducted using a semi-structured questionnaire (Annexure 1) that was developed for this study. The questionnaire included information on how the peoples differentiate between high-quality and chemically contaminated dried fish, what are the reasons for not taking dried fish and perceived health issues following consumption of dried fish and fishery products. The prepared questionnaire was applied for pre-testing among the randomly chosen respondents residing in coastal belt areas (n = 20) to ensure its applicability, clarity, and interview time requirements. The findings of the preliminary testing were excluded from the final analysis. Through the coding of questionnaires, respondents’ responses were kept anonymous. It took 10–15 min to complete each interview.

2.3. Sample collection and processing

Five dried fish species such as Bombay duck (Harpadon nehereus), Ribbon fish (Trichiurus haumela), Chinese promfret (Stromateus chinensis), Silver jewfish (Johnius argentatus) and Shrimp (Penaeus monodon) were collected from the selected fish drying centers (n = 5) for the determination of organoleptic, nutritional and microbial quality. Collected samples were in polyethylene pouch and transferred to the “Seafood Processing, Safety and Quality Control Laboratory” of Department of Fisheries Technology, Patuakhali Science and Technology University, Bangladesh. Samples were further repackaged in vacuum packaging machine (Hualian Machinery Group, Model: HVC-510F/2A-G 20 m³, China) and stored in the freezer until subsequent analysis.

2.4. Sensory quality assessment of dried fish samples

Ten trained panelists (4 male and 6 female) evaluated the sensory characteristics in terms of color, odor, texture, flavor, infestation, general appearance, and acceptability of dried fish. The sensory quality determined using the approach of Mithun et al. (2021) based on defect characteristics of dried fish (Annexure 2). The average defect points <2 was graded as A category (i.e., dried fish were excellent, highly acceptable), average scores between 2 and <5 graded as B category (i.e., good/acceptable), and finally scores ≥5 graded as C category (i.e., rejected; Table 1). The sensory evaluation of this study complies with all regulations and procedure followed by the IFST Guidelines for Ethical and Professional Practices for the Sensory Analysis of Foods (2020). The study design and protocols were examined and permitted by the Research and Training Center (RTC) of Patuakhali Science and Technology University, Bangladesh [Ref. No.: PSTU/RTC/21/22(3); Date: 22.04.2021]. The panelists were trained and demonstrating the appropriate procedure of sensory evaluation of dried fish. All test procedures were executed in a manner that minimizes any risk or harms to the soundness of the panelists. The participation was deliberative, and written consent was obtained from all the panelists.

2.5. Proximate composition analysis of dried fish

2.5.1. Determination of moisture content

Moisture content of dried fish was determined by following Association of Official Analytical Chemists (AOAC, 2000) method of proximate analysis using hot air oven (DO-35, Human Instrument Co. Ltd, Korea) at 105 °C for 16 h. The percent of moisture was calculated as the following Eq. (1):

\[
\text{Percent of (\%) moisture} = \frac{\text{Weight of sample with crucible} - \text{Weight of dried sample with crucible}}{\text{Weight of sample}} \times 100
\] (1)

2.5.2. Determination of protein content

The protein content (N₂) of the dried fish was determined following AOAC (2000) guideline by Kjeldahl apparatus (Buchi, CH-9230, Switzerland). The whole procedures were divided into three parts such as digestion, distillation and titration. Finally, percent of protein was calculated using the following Eq. (2):

\[
\text{Protein content (\%) = \% of N₂ content \times Protein conversion factor (6.25)}
\] (2)

Here, % of N₂ content: Burette reading × Normality of H₂SO₄ (0.2) × ml equivalent of N₂ (1.4).

2.5.3. Determination of lipid content

The lipid content of the dried fish was estimated according to AOAC (2000) method (with some slight modification) by Soxhlet apparatus (LABORTECHNIK GmbH Co. Ltd, Germany) using acetone as a solvent. Lipid content (%) was calculated by the following formula (3):

\[
\text{Lipid content (\%) = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100}
\]
2.5.4. Determination of total ash content

Total ash content was determined following AOAC (2000) method by using muffle furnace (Huanghua Faithful Instrument Co. Ltd, China) at 550 °C for 6 h. The ash content was calculated by the following Eq. (4):

\[
\text{Ash content} \% = \frac{\text{Weight of ash with crucible} - \text{Weight of empty crucible}}{\text{Weight of sample}} \times 100
\]

2.6. Microbial study of dried dish

For the microbiological study, we investigated one dried fish (i.e., dried Bombay duck) that was obtained from three fish drying centers in Bangladesh: Chittagong, Cox’s Bazar and Bhalia. As dried Bombay duck has higher consumer acceptability and widely consumed species in Bangladesh (Hoque et al., 2021; Hossain et al., 2013), its safety and quality need to be evaluated. For this reason, we chose the species for microbiological investigation.

2.6.1. Preparation of sample

Approximately one (1) gram of sample was blended appropriately with 9 ml of sterile 1.5% peptone water. Thus a 1:10 dilution of the samples was obtained. The mixtures were centrifuged (centrifugation machine model: NF800R, Nave, Australia) at 5000 rpm for 10 min, and the supernatants were taken in sterile test tubes. After that, 1 ml of the supernatant was used to dilute the sample 10 times in 0.9% normal saline.

2.6.2. Enumeration of total plate count in dried fish sample

Total plate count (TPC) of dried fish sample (Bombay duck) was performed in nutrient agar (HiMedia, India) media following the APHA (1992) protocol. The media was made by liquefying 28 g of nutrient agar in 1000 ml of distilled water. Then, the nutrient agar media was sterilized at 121 °C for 15 min in the autoclave. The developed medium was poured into sterile petri dishes for incubation before being cooled at 45 °C. Finally, inoculated plates were incubated at 37 °C for 24 h and the colonies between 30 and 300 were considered for TPC counts. The TPC counts were presented as colony forming units per gram (cfu/g).

2.6.3. Enumeration and detection of pathogenic bacteria (E. coli, Salmonella sp. and Vibrio sp.)

Dried fish sample was enriched with peptone water, selenite broth and alkaline phosphatase for detection of E. coli, Salmonella sp. and Vibrio sp., respectively by following the instruction of manufacturer (Hi-media, India). The E. coli, Salmonella sp. and Vibrio sp. in dried fish sample were enumerated using eosin-methylene blue (EMB) agar, xylose lysine deoxycholate (XLD) and thiosulphate citrate bile salt sucrose (TCBS) agar, respectively by following the protocols of APHA (1992). After the recommended hours of incubation (generally at 37 °C for 24–72 h), colonies were counted. We observed green metallic sheen colonies, black centered colonies and yellow colonies for total E. coli count (TEC), total Salmonella sp. count (TSC) and total Vibrio sp. count (TVIC), respectively. Colony ranges 30 to 300 were considered to calculate bacterial load and the results expressed as cfu/g.

When the microorganisms displayed characteristics colony on selective media using spread plate method, additional validation was carried out by streak plating, which involved examining the discrete colony and color characteristic. When the desired microorganism were identified based on colony characteristics, then biochemical tests including indole test, methyl-red (MR) test, Voges Proskauer (VP) test, citrate utilization test, catalase test, and oxidase test of microorganisms were performed for presumptive identification of the respective pathogenic microorganisms (either E. coli, Salmonella sp. and/or Vibrio sp.) (AOAC, 1998).

2.7. Data analysis

The statistical software called Statistical Package for Social Sciences (SPSS) (IBM version 23.0, Armonk, NY, USA) was used to analyze the data. For the purpose of summarizing the relevant variable, descriptive statistics (i.e., measures of central tendency, variability and frequency distribution) including responses, percentage, mean, and standard deviation were computed. A one-way analysis of variance (ANOVA) and Duncans’ multiple range test were employed to assess the mean comparisons. Finally, statistical significance was defined as p-values <0.05.

3. Results and discussion

3.1. Sensory quality of collected dried fish

Table 2 represents the sensory quality of common dried fish collected from different major fish drying centers in Bangladesh. For general appearance, all the dried fish species from Cox’s Bazar and Chittagong were excellent or good, thus the overall acceptability was higher/excellent (lower score), while samples from the other three regions had good or moderate appearance indicating moderately acceptable/good. Overall, among all the dried fish studied, Bombay duck, shrimp and Ribbon fish had the higher acceptability than Silver jewfish and Chinese pomfret (Table 2).

The variation in sensory quality of dried fish might be due to the differences in natural composition of the respective dried fish. Naturally, Bombay duck and shrimp contain higher water content in its raw state than other species studied here, where Chinese pomfret and Silver jewfish contain high flesh, fat and bone consequences of different physical state obtained upon drying. Thus, this variation in sensory quality of all dried fish resulted from its natural constituent, processing, packaging and storage conditions applied.

3.2. Nutritional quality of dried fish

Based on nutritional results (Table 3), an reverse relationship was observed between moisture and protein content of dried fish. Shrimp (60.08 ± 1.47 to 64.33 ± 0.99%) had significantly higher protein content than dried Bombay duck (51.80 ± 0.95 to 55.61 ± 1.10%) and Ribbon fish (52.13 ± 1.33 to 62.63 ± 1.20) (p < 0.05). Similar trends of changes in moisture content (in relation to protein content deviation) were found for all the respective dried fish species. The result was in-accordance with the protein content 51.98–58.33% in dried Bombay duck (Siddique and
Regarding the ash content of different site in a specific location (Majumdar et al., 2017), 5.98% (Siddique and Aktar 2011; Majumdar et al., 2017). The lipid level was varied from 5.86% to 7.78% in dried Bombay duck (Siddique and Aktar 2011), 5.98%–6.81% (Majumdar et al., 2017), and 4.20%–13.03% in different dried fish (Rana et al., 2020). The amount of lipid in dried fish may alter according to biological state like species, age and maturity of the fish, and also seasons of fishing and drying the respective species (Pigott and Tucker 1990; Majumdar 2017). Irrespective of dried fish samples and locations, this study revealed the ash content ranges from 13.89 ± 0.94% to 20.07 ± 1.64% (Table 3). The observed ash content was also comparable with the reported value from 15.87 to 32.22% for different marine dried fish including Bombay duck and ribbon fish (Paul et al., 2018), 11.21%–28.15% in five different dried fish (Flowra and Tumpa 2012), and 20.06 ± 0.36% in dried Bombay duck (Pravakar et al., 2013).

The result revealed that fish species variation appears to have a higher influence on the nutritional variation of the resulting dried fish than regional differences. Between shrimp and fish, the difference in nutritional content might result from biological differences, where shrimp is a crustacean containing shell and less muscle but the other two species belong to the finfish group having higher muscle/flesh. In addition, nutritional variation in dried fish from different geographical locations might be due to differences in raw materials sourcing, processing and processing techniques, and relative humidity in the drying yard, and hygiene and sanitation practices during drying. Based on the nutritional composition, the studied dried fish in the current study indicated with the higher nutritive value in terms of protein, fat and mineral contents, and its higher consumption could contribute to the nutritional security of the country (Siddhath et al. 2020).

Considering the availability, consumer’s preference, consumption amount and nutritional content, the dried Bombay duck from the higher consumption areas (Chittagong, Cox’s Bazar and Bhola) was selected for further microbiological study.
Quantitative microbiological results of dried Bombay duck from three different locations were associated with TPC in the sample from respective sites. The observed results (higher microbial count in the sample from Cox’s Bazar and lower from Chittagong) were correlated with the higher moisture content of the sample (Bombay duck). All of the shrimp isolates from dried Bombay duck, Shrimp and Ribbon shrimp were highly acceptable. Thus, further attention is required to reduce microbial contamination. Consumer perceptions regarding the quality of dried fish during purchase (Krishnal and Dayaani 2014).

Among those who considered different characters of dried fish, only 2.3% considered appearance to identify the quality of dry fish, mostly (26%) considered color as an indicator to identify the goodness of dry fish, and 18.3% considered texture and odor, respectively. When asked if they would purchase dry fish from different areas, they mostly (26%) considered color as an indicator to identify the goodness of dry fish. To identify the quality of dry fish, only 10.2% and 6.3% of participants were considered texture and odor, respectively. When the same dried fish and the same constituent; different capital alphabet in the same column represent significant difference (p < 0.05) between different locations for the same dried fish species for the same location and constituents. Bold and italic values indicates highest and lowest values of that particular nutrient.
looking at the specific location of the study area, color was mostly considered in Patuakhali (41.5%) to identify whether the dry fish is good or free of chemical contamination. But the appearance (0%) of dry fish was not totally considered in Cox’s Bazar, Patuakhali and Khulna regions to identify their quality (Figure 2). Organoleptic the quality parameters of dried fish such as color, odour, taste, tactile, appearance etc. can be evaluated by human senses (Martinsdottir et al., 2009), which is important for determining dried fish quality by itself. Therefore, consumer knowledge and practices regarding sensory-quality aspects of dried fish should be increased to identify better quality dried fish during buying. A previous study reported that 70%, 66.67% and 58.33% of consumers considered the appearance, colour and smell of dry fish when purchasing, respectively (Krishnal and Dayaani 2014).

3.5. Reasons for not consuming dried fish and perceived health problems

Of 500 households, 116 households (23.2%) reported that they didn’t consume dry fish and the major barriers to consuming the dry fish were – (i) do not like dry fish, and (ii) don’t like the smell of dry fish. Besides, many participants also reported that dry fish is much expensive and made from spoiled fish, that’s why they don’t consume it (Figure 3).

Figure 2. Consumer considered characters to identify good and chemically contaminated dried fish in the study area.

Figure 3. Reasons for not consuming dried fish (n = 116).
Among the participants who were used to consuming dry fish (n = 384), 3.13% of them reported that they faced an acidity problem after consuming dry fish, while 1.8% and 1.05% reported diarrhea and vomiting problems, respectively. In the study, another 94.8% of respondents reported no complications after consuming dry fish (Figure 4). Majority of the respondents faced no health complications after consuming dried fish, which indicates the better quality and safety of dried fish. However, several health complications such as acidity, diarrhea and vomiting can be occur rarely due to consumption of contaminated dried fish either by microbial or heavy metals or DDT (Bhuiyan et al., 2009a, Bhuiyan et al., 2009b; Paul et al., 2018; Rahman et al., 2019).

**4. Strengths and limitations of the study**

The current research has several strengths. This study assessed the nutritional properties and quality aspects of mostly consumed dried fish species and compared the qualitative features across the regions of Bangladesh, which contribute to the growing body of literature. Findings of this study may inform policymakers and government authorities to develop and implement an evidence-based interventions and food safety awareness programs to improve the quality and safety aspects of dried fish. This research also indicates where to start (e.g., we demonstrated that dried fish from which region had the highest or lowest quality) the intervention to improve the food safety and hygiene practices among the dry fish workers and which dried fish species should be encouraged to consume. Moreover, the strength of this study lies in robust study design such as both laboratory analysis and cross-sectional survey with appropriate statistics. However, this study has some shortcomings and to overcome those, further studies recommended molecular identification of pathogenic microorganisms, biochemical analysis like TMAO, TVBN, PV and TBARS during storage of dried fish. For survey data, self-reporting bias may be occur.

**5. Conclusions**

In conclusion, our study revealed a higher nutritive value or nutritional quality of dried fish. But, the poor microbiological condition (higher TPC, and presence of Vibrio sp.) in dried Bombay duck may be caused by unhygienic and unsanitary handling by labor/processors, conventional processing techniques, improper storage and packaging of the products. Thus, we need to focus on maintaining the qualitative and quantitative aspects of microbial quality and safety of the dried fish for the public health benefits. In aims to meet-up the higher consumer demands and to sustain goodwill in the competitive market, special attention is now needed for maintaining improved processing, handling and storage, and proper personal hygiene and sanitation conditions. Therefore, the authorities should implement effective preventive and control actions by educating and training of producers/processors, ensuring modern fish drying technologies, quality raw materials, pure water, packaging and storage conditions on safe dried fish production, and raising consumer’s knowledge of food safety to get safe and nutritious dried fish across the country.

**Declarations**

**Author contribution statement**

Md. Hasan Al Banna; Fatima Tamanna; Md. Mahmudul Hasan: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Md. Sazedul Hoque: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Pronoy Mondal: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Md. Belal Hossain: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

Suprakash Chakma; Mst. Niloy Jaman; Md. Abu Tareq: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Md Shaifiquel Islam Khan: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

**Funding statement**

Dr. Md. Sazedul Hoque was supported by International Foundation for Science, Sweden, Organisation for the Prohibition of Chemical Weapons, Netherlands [I-3-E/5670-2] for consumer survey and sample collection. In addition, nutritional and microbial laboratory analysis was supported by the Bangladesh Academy of Sciences-United States Department of Agriculture Endowment Program (4th phase BAS-USDA PSTU FI-17).

**Data availability statement**

Data will be made available on request.

**Declaration of interest’s statement**

The authors declare no conflict of interest.
Supplementary content related to this article has been published online at https://doi.org/10.1016/j.heliyon.2022.e10830.

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