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Nutrition and quality status of dry-salted Hilsa (*Tenualosa ilisha*) in the retail market of Bangladesh

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Abstract

Hilsa (*Tenualosa ilisha*) is the national fish and geographical indication (GI) product of Bangladesh. Salting is the most suitable and oldest method of Hilsa preservation in Bangladesh. The quality of the traditionally salted dried fish in retail markets of Bangladesh is doubtful. This study aimed to assess the organoleptic, nutritional, biochemical, and safety attributes of dry-salted Hilsa. Organoleptic characteristics, nutritional (proximate composition, salt content), biochemical (pH, total volatile base-nitrogen (TBV-N), thiobarbituric acid reactive substances (TBARS)), and safety attributes (total plate count, presence of coliform bacteria, *Salmonella-Shigella*, *Staphylococcus,* enteropathogenic *Vibrio*, and heavy metals: Hg, Cd, Cr, Pb, Ni, Cu, Zn) of dry-salted Hilsa was analyzed by standard methods. The product was organoleptically acceptable (defect point score: 2.65). Moisture (48.16%), protein (29.95%), lipid (4.36%), ash (17.53%), and salt (17.90%) content showed that the product was highly nutritious for human consumption. The pH, TVB-N, and TBARS values were 6.21, 83.25 mg N/100 g, and 4.18 mg MDA/Kg, respectively. Total plate count, coliform, *Staphylococcus* bacteria were 7.87×10^4, 6.96×10^3, and 2.70×10^3 CFU/g, respectively, indicating that the microbiological quality of the product was not satisfactory. However, *Salmonella-Shigella* and Enteropathogenic *Vibrio* were not found in the samples. Amounts of Hg, Cd, Cr, Pb, Ni, Cu, and Zn were 0.00005, 0.00004, 0.00112, 0.00102, 0.00134, 0.00167, and 0.00045 µg/g, respectively; which were within safety limit for human consumption. To improve the quality of dry-salted Hilsa, processors, sellers, and other stakeholders should be trained up properly.

1. Introduction

Fish are recognized as highly perishable food items because of their high water activity, neutral pH, and the presence of autolytic enzymes (Ashie *et al*., 1996). Various processing methods such as drying, salting, smoking, canning and more are employed to preserve fish (Nowsad, 2007). Salting has been used since time immemorial and salting along with drying was the only widely available method of preserving fish until the 19th century. Dried and salted fish products are an important part of the diet in the Caribbean, North Africa, Southeast Asia, Southern China, Scandinavia, coastal Russia, and the Arctic (Nowsad, 2007).

About 85% of world Hilsa (*Tenualosa ilisha*) production occurs in Bangladesh. Hilsa contributes to 12% of total fish production in Bangladesh. Hilsa is the national fish and geographical indication (GI) product of Bangladesh (Matin and Shamim, 2018). In 2014, the value of total Hilsa production was BDT 250 billion, which contributed 1% to the national GDP (DoF, 2014). From 2018 to 2019, Bangladesh harvested 0.53 million MT Hilsa, about 1.5 times higher production than in 2014 (DoF, 2020). The unique taste of Hilsa has often been attributed to the presence of a higher amount of fatty acids e.g. stearic acid, oleic acid and polyunsaturated fatty acids (ω3, ω6) *viz*. linoleic, linolenic, arachidonic, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Mohanty *et al*., 2011). Unlike other fishes, drying or freezing is not suitable for fatty fishes like Hilsa due to lipid oxidation and rancidity, and consequent protein deterioration (Hultin,

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1988). Salted fish has a universal appeal to the consumer for its characteristic flavour and colour. Salting is the most efficient and oldest method of Hilsa preservation in Bangladesh. The salted Hilsa is locally known as 'lona ilish' or 'nona ilish'. The product found in Chandpur, Noakhali, and Barishal regions are dry, wet, or mixed salted. However, partial anaerobic salt fermentation is practised in Chittagong, and Cox's Bazar region in Bangladesh (Nowsad, 2007).

Bangladesh exported 166 MT of salted-dehydrated fish products worth BDT 0.19 billion from 2018 to 2019. However, export earnings from salted-dehydrated fish products have been gradually decreasing (DoF, 2020). It has been reported that the quality of the traditionally salted dried fish available in the local markets is not satisfactory for human consumption (Mustafa *et al.*, 2012; Rohomania *et al.*, 2014; Mansur *et al.*, 2017; Hosen *et al.*, 2018). Spoilages of salted fish are notably pink spoilage, dun spoilage, reddening, salt-burn and rancidity of lipids that can result in significant economic losses (Nowsad, 2007). Moreover, intake of highly oxidized fish products may cause adverse effects on the human body such as ageing, heart disease, cancer and brain dysfunction (Kinsella, 1987; Rasul *et al.*, 2020; Rasul *et al.*, 2021). It is important to understand how much these salted products are nutritious and safe for human consumption. Therefore, this study aimed to investigate the organoleptic, nutritional, biochemical, and safety attributes of salted Hilsa available in the retail market of Bangladesh.

2. Materials and methods

2.1 Dry-salted Hilsa sample

Ten dry-salted Hilsa samples (average weight 500 ± 200 g) were collected from the Mirpur market, Dhaka, Bangladesh, and transported to the Fish Processing Laboratory at Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh in sterilized polyethylene bags, and 5 fishes were kept at ambient temperature (22-30°C) for organoleptic and microbial analysis, and rest 5 fishes were kept at -18°C for other experimental purposes.

2.2 Sensory assessment

Sensory quality (appearance, colour, odour, texture, cleanliness and overall quality) of salted Hilsa was determined by 10 expert panelists according to the sensory characteristics and relevant defect points given by Mansur *et al.* (2017), where the score of defect points (SDP) was calculated using the following equation. To prevent outside smell, laboratory doors and windows were kept closed during the examination.

$$SDP = \frac{\Sigma DP}{n}$$

Here, SDP = score of defect points, ΣDP = summation of defect points, and n = number of characteristics.

2.3 Nutritional content analysis

Proximate composition (moisture, crude protein, crude lipid and ash) of salted Hilsa was analyzed according to the AOAC (2006) methods. Modified Mohr's method was followed for the determination of per cent (%) NaCl (Dharshini *et al.*, 2018). In brief, 1 g of salted fish sample was powdered and dissolved in 30 mL of boiling distilled water. Then 1 mL of 5% potassium chromate solution was added as an indicator and titrated against 0.1 N silver nitrate solution. A red-brown precipitate of silver chromate indicated the endpoint.

2.4 Biochemical analysis

pH value was measured by homogenizing 10 g of fish muscle with 90 mL of distilled water, and the pH was directly measured using a pH meter (Jenway 3510, UK). Total volatile base nitrogen (TVB-N) was determined following the method of AOAC (2006). Thiobarbituric acid reactive substances (TBARS) were measured according to the method described by Buege and Aust (1978). TBARS was quantified by preparing a standard curve using 1,1,3,3-tetraethoxypropane at different concentrations ranging from 0 to 10 ppm.

2.5 Microbial quality and heavy metal analysis

Microbiological analysis was performed according to the methods described by Ben-David and Davidson (2014), and Seeley and Vandemark (1981). In brief, 25 g of salted fish was transferred aseptically to a stomacher bag, and 225 mL of 0.85% of sterile saline water was added and homogenized for 60 secs in a Stomacher® 400 Circulator (UK). The stock solution was then serially diluted logarithmically in test tubes with 0.85% of sterile saline water, cultured in plate count agar (HiMedia, India) and incubated at 37°C for 24 hrs. Eosin methylene blue (EMB) agar, *Salmonella*-*Shigella* (SS agar), Mannitol salt agar (MSA) and Thiosulfate-citrate-bile salts-sucrose (TCBS) agar of HiMedia, India were used to isolate and enumerate coliform bacteria, *Salmonella*-*Shigella*, *Staphylococcus* and enteropathogenic *Vibrio*, respectively. Heavy metals such as Mercury (Hg), Cadmium (Cd), Chromium (Cr), Lead (Pb), Nickel (Ni), Copper (Cu), and Zinc (Zn) content were analysed by inductively coupled plasma mass spectrometer (ICP-MS) (NexION 1000, PerkinElmer). The calibration standards were prepared from 100 ppm ICP multi-element standard solution

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(Merck Millipore, Germany).

2.6 Statistical analysis

For each parameter, the analyses were repeated 3 times. All data are expressed as mean ± standard deviation.

3. Results and discussion

3.1 Organoleptic characteristics

Organoleptic characteristics of dry-salted Hilsa are presented in Table 1. The dorsal part of the body was slightly blackish and the ventral part was yellowish (Figure 1). Characteristic salty dry fish colour and odour were observed in this product. The texture of the sample was hard, with no elasticity. There was no visible sign of spoilage. The score of the defect point was 2.65, which indicates that the product was acceptable for human consumption. Rahman *et al*. (1999) observed that salted Hilsa become slightly rancid within 1-1.5 months of storage at room temperature. According to Mansur *et al*. (2017), sensory characteristics of salted river shad Hilsa were a light yellow colour, soft texture, cheesy salted flavour, no broken piece, and the score of defect point was 1.8, the overall quality of that salted product was excellent. The result of this study is more or less similar to Mansur *et al*. (2017), however, some variations were observed, which might be due to differences in sample processing, handling and storage.

Figure 1. Dry-salted Hilsa

3.2 Nutritional content

The proximate composition and salt content of salted Hilsa has been presented in Figure 2. Moisture, crude protein, crude lipid and ash content of salted Hilsa was 48.16%, 29.95%, 4.36% and 17.53%, respectively on a fresh matter basis. The moisture content of salted Hilsa was almost similar to that reported by Kaisar *et al.* (2017), who found that the moisture content varied from 44.91%-48.18% in dry-salted Hilsa samples obtained from 3 different regions in Bangladesh. Similar results were also observed by Mustafa *et al.* (2012), and Hosen *et al.* (2018). Moreover, the variation of moisture content depends on temperature, humidity, season, time of storage and type of fish (Sivashanthini *et al.*, 2012). Moisture content influences the growth and flourishment of the microorganisms in salted dried fish and causes microbial spoilage (Majumdar *et al*., 2018; Rasul *et al*., 2019).

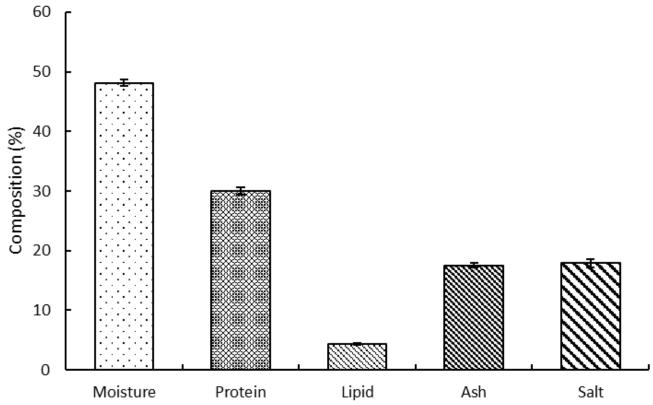

Figure 2. Proximate composition and salt content of dry-salted Hilsa. The error bars represent mean ± SD of triplicates.

The crude protein content observed in this study was comparatively higher than those reported by Hosen *et al.* (2018), who reported 21.91-23.58% protein content in salted Hilsa. Mustafa *et al.* (2012) observed that protein content varied from 25.09% to 36.24% in salted Hilsa prepared by various salting methods. This is due to the loss of water, which increase other forms of non-protein nitrogen in the muscle tissue of salted fish (Mustafa *et al.*, 2012).

In this study, the lipid content of salted Hilsa was comparatively lower because the fish contained eggs, which suggests that the fish was caught in the spawning season. During the spawning season, the lipid content of the fish body remains low. Mustafa *et al.* (2012) found that the lipid content of salted Hilsa varied from 4.65% to 6.81%, while the lipid content in fresh Hilsa was 9.49%. A decrease in the lipid content in salted fish products might be due to the leaching out of lipids from fish tissue and tissue compaction for osmotic pressure (Binici and Kaya, 2006). Ash content found in salted Hilsa was more or less similar to that reported by Hosen *et al.* (2018), who reported that ash content in salted Hilsa ranged from 16.15% to 17.55%. Ash content is a measure of the total amount of minerals within the food and is considered an indicator of digestibility (Sivashanthini *et al.*, 2012).

Table 1. Organoleptic characteristics and general cleanliness of dry-salted Hilsa

Appearance	Colour	Odour	Texture	Cleanliness	Overall quality
Salted Hilsa like appearance, fish cut into chunks, dry	Yellowish, brownish	Dry fish like, rancid	Tough	Presence of sand	Acceptable

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The average value of salt content in salted Hilsa was 17.90%. Kaisar *et al.* (2017) found 17.05-19.04% salt content in salted Hilsa. By reduction of water activity (a_w) in a food system, salt renders a condition less favourable for microbial growth and thus guarantees the quality and stability of the finished salted product (Lück and Jager, 1997). In general, foodborne pathogenic bacteria become inhibited by an a_w of 0.92 or less, which is equivalent to a salt concentration of 13% (w/v) (Eyo, 1991). However, adding a large amount of salt may cause some adverse effects on human health e.g. increased blood pressure, and damage internal organs (Sivashanthini *et al.*, 2012). The salt content in fish muscle enhances the oxidation of highly unsaturated lipids (Aubourg and Ugliano, 2002). Salting and drying processes are key factors that contribute significantly to the reduction of total viable counts, moisture contents, and a_w in fish products (Majumdar *et al.*, 2017; Rasul *et al.*, 2018).

3.3 Biochemical indices in dry-salted Hilsa

pH, TVB-N, and TBARS values as indicators of spoilage in dry-salted Hilsa have been presented in Table 2. pH is a good indicator for the determination of freshness of fish and the extent of microbial spoilage (Huss, 1995). In this study, the average value of pH in the dry-salted Hilsa sample was 6.21. Majumdar and Basu (2010) found that the pH of salted Hilsa was 5.66. The reason for the low pH value of the product may be attributed to the fact that the samples were undergoing fermentation and were not spoiled. Again, the low pH value can be explained by the increase in the ionic strength of the solution inside the cells (Goulas and Kontominas, 2005).

Table 2. Condition of spoilage in dry-salted Hilsa

Parameters	Results
pH	6.21±0.05
TVB-N	83.25±0.59 mg N/100 g
TBARS	4.18±0.22 mg MDA/Kg

Values are presented as mean ± SD (n = 3).

The average TVB-N content found in the dry-salted Hilsa sample was 83.25 mg N/100 g. TVB-N value found in this study is lower than the recommended acceptable value (100–200 mg/100 g) for a variety of salted and dried products (Connell, 1980). Majumdar and Basu (2010) found a comparatively lower amount of TVB-N content (48 mg N/100 g) in dry-salted Hilsa. It has been reported that fish sold in the retail market contain TVB-N as high as 98 mg N/100 g (Iyer *et al.*, 1986). The level of TVB-N compounds e.g. ammonia and trimethylamine produced by bacterial and endogenous enzymes are used as a spoilage indicator for fish and fish products (Gram *et al.*, 2002). To some extent, these compounds impart the typical flavour and aroma of the final product (Majumdar and Basu, 2010).

TBARS is a measure of malonaldehyde (MDA), which is an end-product of lipid oxidation (Gomes *et al.*, 2003). TBARS value found in this study was 4.18 mg MDA/Kg. According to Connell (1990), TBARS value of 1-2 mg MDA/Kg is usually regarded as the safety limit beyond, which an objectionable odour/taste is developed. So, the products were highly oxidized. Hilsa is a fatty fish, therefore, it is usual to find high TBARS content in salted Hilsa. Moreover, direct exposure to air, sunlight as well as higher storage temperature accelerate lipid oxidation in the fish product.

3.4 Microbial quality and heavy metal content in dry -salted Hilsa

Total plate count, coliform bacteria, *Salmonella-Shigella*, *Staphylococcus*, and Enteropathogenic *Vibrio* has been enumerated in Table 3. Coliform bacterial load was the highest among all the bacterial counts. *Salmonella-Shigella* and Enteropathogenic *Vibrio* were not found in the salted Hilsa sample. The quality of salted and sun-dried fish is often adversely affected by microbial contamination (Aktar *et al.*, 2018). The maximum recommended bacteria count for the good quality fish product is 5.0×10^5 CFU/g (ICMSF, 1986), while 1.0×10^7 CFU/g for the marginally acceptable quality fish product (Odebiyi *et al.*, 2013). In this study, the total plate count and coliform bacteria were 7.87×10^4 CFU/g and 6.96×10^3 CFU/g, respectively. Sultana *et al*. (2008) found 1.4×10^2-7.4×10^3 CFU/g total coliform in salted Hilsa. The presence of coliform in food is linked with inadequate hygienic practices, mishandling, improper storage and the use of dirty water in ice (Ekici and Dümen, 2019). Microbial contamination of fish depends upon the source of fish, pH, temperature, the acidity of water, nature of handling, processing time elapsed between harvesting and marketing and water used during marketing. Rohomania *et al*. (2014) found 2.10×10^3, 4.00×10^4, 3.20×10^3, and 1.50×10^2 CFU/g *Staphylococcus* in salted Hilsa samples collected from 4 different markets of Dhaka city in Bangladesh. Therefore, the *Staphylococcal* count found in this study is consistent with the finding of Rohomania *et al*. (2014). No *Salmonella* and *Vibrio* were detected in this study. The possible reason behind the absence of *Salmonella* in this study is that there was no *Salmonella* infection in humans associated with Hilsa salting and marketing, and also there was no environmental contamination (U.S. Food and Drug Administration, 2020). Generally, *Vibrio* are an inhabitant of the marine environment, this might be the reason for the absence of *Vibrio* in the examined samples that remained in an inland environment (Venkateswaran, 1999). Shamsuzzaman *et al*. (2011) also did not find any *Salmonella* and *Vibrio* in salted

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Hilsa.

The minute amount of Hg, Cd, Cr, Pb, Ni, Cu, and Zn were present in salted Hilsa. Heavy metal content in salted Hilsa were 0.00005 µg/g Hg, 0.00004 µg/g Cd, 0.00112 µg/g Cr, 0.00102 µg/g Pb, 0.00134 µg/g Ni, 0.00167 µg/g Cu and 0.00045 µg/g Zn (Figure 3). Heavy metal contents were found in the order of Cu >Ni >Cr >Pb >Zn >Hg >Cd. The maximum allowable limit (MAL) or permissible limit for Hg, Cd, Cr, Pb, Cu, and Zn is 0.15 (US EPA, 2021), 0.05 (WHO, 2011), 0.05 (FAO/WHO, 1989; WHO, 2011), 0.5, 30, and 40 (FAO/WHO, 1989) µg/g, respectively. No data on MAL of Ni was found in European Community (EC, 2005), FAO (Nauen, 1983), and FAO/WHO (1989). Therefore, all the heavy metals found in salted Hilsa were within the acceptable level for human consumption. Mansur *et al.* (2017) also found an insignificant amount of Cd, Cr, Pb, Cu, and Zn in salted Hilsa. The source of heavy metals in Hilsa is believed to be due to water pollution by wastes discharged from fertilizer and tannery industries.

Table 3. Bacterial count in dry-salted Hilsa

Microbes	Count (CFU/g)
Total plate count	$7.87\pm0.07\times10^4$
Coliform bacteria	$6.96\pm0.97\times10^3$
Salmonella-Shigella	0.00
Staphylococcus	$2.70\pm0.62\times10^3$
Enteropathogenic *Vibrio*	0.00

Values are presented as mean ± SD (n = 3).

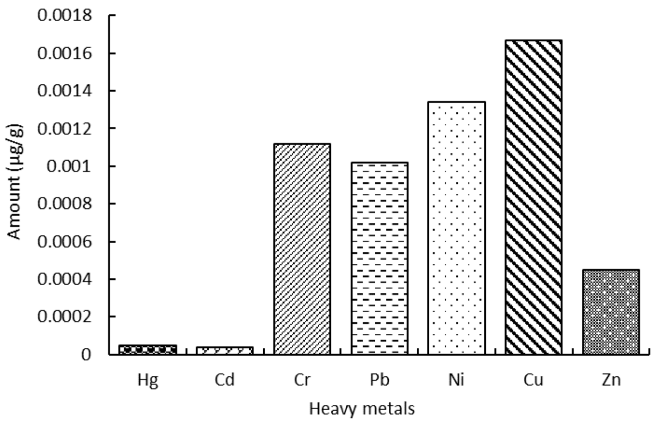

Figure 3. Concentrations of different heavy metals (Hg, Cd, Cr, Pb, Ni, Cu, and Zn) in dry-salted Hilsa (values are too small, hence standard deviation cannot be applied).

4. Conclusion

The sensory characteristics such as colour, odour and texture of salted Hilsa revealed that the product was of good quality. Proximate composition showed that the product was highly nutritious for human consumption. Total volatile basic nitrogen content was comparatively higher but remained within the acceptable limit. However, the TBARS value revealed that the salted Hilsa was highly oxidized. The microbiological quality was not so satisfactory. Therefore, consumption of this product sometimes might cause food poisoning and health risks. All the heavy metals found in the salted Hilsa were within the safety limit. Conventional salting maintains good organoleptic characteristics, though biochemical and microbiological indices are a matter of concern. To improve the quality of salted Hilsa, based upon the results of this study, the local fishing communities, processors, sellers, and other stakeholders should be trained properly through the collaborative efforts of government agencies and NGOs.

Conflict of interest

The authors declare no conflict of interest.

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