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Nutritional Evaluation of Raw, Curried, and Fried Forms of Three Local Fish Species in Bangladesh: Implications for Public Health

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ABSTRACT

The study examines the effects of culinary techniques on the organoleptic, nutritional, biochemical, microbiological, and heavy metal content of three commonly consumed fish species: *Tenualosa ilisha* (Ilish), *Putius chola* (Puti), and *Labeo rohita* (Rui). The curry samples of each fish exhibited excellent organoleptic quality, whereas the fresh samples had a lower moisture content ($p < 0.05$). Fried pieces had higher ash, protein, lipids, and carbohydrates ($p<0.05$). The culinary technique negatively affected the fried samples, leading to higher nutrient loss ($p < 0.05$), a higher per-oxide value (POV) ($p < 0.05$), and a higher total volatile base of nitrogen (TVBN) compared to raw and curry fish, as well as higher heavy metal retention ($p < 0.05$). As the Ni content of each fish item exceeds the permitted limit and the Cr content exceeds the limit in only Rui fish items, according to the WHO/FAO recommended maximum permissible limit, this creates concern for public health. In microbiological analysis, there is no significant difference between curry and fried items. In conclusion, the study demonstrated that curry fish was the most effective item in terms of culinary technique for retaining higher nutrients, nutritious compounds, and lower POV and TVBN values compared to other fish items.

1. Introduction

Fish is a key source of animal protein and healthy lipids (Luthada-Raswiswi et al., 2021; Tacon & Metian, 2013) and currently provides 17% of all the protein consumed worldwide (FAO, 2022). Fish is an excellent source of nutritional elements, providing a significant amount of proteins as well as a wide variety of fats (ω-3 and ω-6 fatty acids), minerals, and vitamins such as vitamins A and D, magnesium, iron, and phosphorus, all of which are necessary for maintaining good health (Khalili Tilami & Sampels, 2018; Balami et al., 2019; Mendivil, 2021). Fish is beneficial in the prevention of malnutrition (McMullan et al., 2023) reduce cholesterol levels, reduce the risk of coronary heart diseases, stroke, preterm diseases, etc. (Bassey et al., 2014; Caldeira et al., 2023; Krittanawong et al., 2021). Fish have played a significant role in meeting dietary requirements and ensuring nutrition security for the growing world (Quaas et al., 2016; Pradeepkiran, 2019).

Bangladesh is an agriculture-based riverine country (Hossain et al., 2023) that has the 3rd largest production in inland capture fisheries and the 5th largest aquaculture production in the world (FAO, 2023; M. M. Alam & Haque, 2021; Saifuddin et al., 2022). Among 250–266 species of freshwater fish (Ferdous et al., 2023; Haque et al., 2024), Ilish (Tenualosa ilisha), Puti (Putius chola), and Rui (Labio Rohita) are very familiar, delicious, and nutritious fish in Bangladesh and also found in India and Myanmar (Shaheen et al., 2013). The above three fish are used as samples in the present study. Generally, punti and rui fish are available throughout the year (DoF, 2019). Ilish fish are known for their unique flavor and taste worldwide (De et al., 2019; Mahmud et al., 2020). These fish have had a strong cultural and culinary heritage for centuries in Bangladesh (Devi & Sarojnalini, 2012). Fish is rarely eaten uncooked, so various cooking techniques must be involved before consumption (Marimuthu et al., 2012).

Cooking is the art of preparing food for consumption by using heat. (Carmody & Wrangham, 2009; Dudeja & Singh, 2017). Cooking methods have a significant impact on the chemical makeup and nutritional value of fish muscle (Erkan et al., 2010; Uran & Gokoglu, 2014). Fish curry and fish fry are highly used culinary techniques in Bangladesh (Bhuiyan et al., 2022). Fish curry is one

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of the important delicacies in Asian restaurants and also a formulated product prepared using spices, herbs, tamarind, coconut paste, and other ingredients to make desired items by nutritional modification (Dayami & Sarojnalini, 2022; Shakila et al., 2012). Frying is one of the oldest ways of food preparation (Oke et al., 2018). Fried fish is cooked by frying with oil, which intensifies the physical features by creating aroma compounds, an appealing look, a coating, and a crispy texture (Bordin et al., 2013; Hosseini et al., 2016). These culinary methods are crucial to reducing harmful microorganisms, enhancing flavor, color, and taste, extending shelf life, nutrient enhancement, enhancing digestability, reducing the loss of nutrients, and producing value-added products (Abraha et al., 2018; Suleman et al., 2020; Zafar et al., 2019). Additionally, it plays an important role in supplying ready-to-cook or ready-to-serve dishes (Adrah & Tahergorabi, 2022).

On the other hand, during this culinary procedure, cooking temperature and time can affect the total quality of the product (Uran & Gokoglu, 2014). These create a major public health concern among people about cooked fish consumption (Sarker et al., 2020; Parvin et al., 2023). But the effect of different culinary methods, quality assessment of cooked items and their comparative study are still lackings in Bangladesh. So, it is crucial to know how much of a positive or negative outcome comes from different culinary items. Therefore, the main purpose of this study was to investigate the impact of various culinary techniques (curry, fry) on the organoleptic quality (color, odor, texture and broken pieces), nutritional quality (nutrient loss), chemical quality (POV and TVBN), microbiological quality, and heavy metal contents, as well as the nutrient losses of ilish, punti, and rui fish items. The study also focused on a comparative study on the quality assessment of raw, curry, and fried fish items based on a public health perspective.

2. Materials and methods

All the reagents and materials used in this study were of analytical grade, reagents purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). The reagents were used without requiring further purification unless stated otherwise.

2.1. Preparation of sample

2.1.1. Collection of fish

For the comparative analysis, the fish (Iilsh, Puti, and Rui) that grow in inland water were collected from the municipal fish market in Kushtia, Bangladesh. These three fish species are some of the more well-liked varieties that are frequently eaten in Bangladesh. The chosen fish were disease-free, fresh and energetic, slightly larger, and purchased at a reasonable price to carry out the investigation. The raw samples were taxonomically recognized prior to collection by Professor Dr. Md. Abdus Samad, Department of Applied Nutrition and Food Technology, Islamic University, Kushtia.

2.1.2. Fish processing and cooking

Fish were typically cleaned to remove scales, slime, and other impurities. Gutting (removing the internal organs) was done with the descaling of the fish, ensuring the fish was ready for further processing. The descaled fish was then cut into similar pieces that were appropriate for cooking and frying, then brushed and cleaned with fresh water. Among the three groups of each fish, one group was kept as raw items, and the other two groups were used for frying (heating in a frying pan with only oil and spices) at 180^{o}C for 10 min and cooking (boiling with water with traditional South Asian spices) 120^{o}C for 25 min, factors are selected based on standard culinary practices in South Asia.

2.2. Food quality of raw, curry, and fried fishes

2.2.1. Organoleptic characteristics

The organoleptic characteristics (colour, odour, texture, and broken pieces) of raw, curried, and fried fish samples were evaluated following the ISO 3972:1991 sensory analysis guidelines. A 5-point hedonic scale was used, where 1 = dislike extremely and 5 = like extremely, to assess each quality parameter. The sensory evaluation was conducted by ten trained panelists, consisting of graduate students from the Department of Food Technology and Nutrition Science. These panelists were not involved in the preparation of the samples to ensure unbiased assessment. Before the evaluation, each sample was cooked and served at ambient temperature in identical, coded containers to eliminate visual bias. Each panelist received the same samples, and questionnaires were distributed for recording their assessments. Panelists were instructed on how to use the hedonic scale and given time to cleanse their palate between samples. This sensory evaluation procedure was adapted and modified from previous studies (Abbey et al., 2019; Nowsad & Hoque, 2021), while the questionnaire format and evaluation method were also guided by Aberoumand et al. (2014).

The grading choice of features and scoring hedonic characteristics and scores for organoleptic test option presented in Table 1.

2.3. Nutritional composition

2.3.1. Determination of moisture content

A porcelain crucible (which had previously been cleaned, heated to around 100°C, and cooled) was used to weigh around two grams of the sample. The crucible containing the sample was heated in a thermostat oven (Gallenkamp, HOTBOX, and Model 306) for 24 h at 105°C. It was then cooled in desiccators and weighed. The process was repeated at an interval of half an hour until two consecutive weights were the same (AOAC, 2005).

$$Moisture\ (\%) = \frac{Sample\ Weight\ before\ drying - sample\ weight\ after\ drying}{Sample\ weight\ before\ drying} \times 100\%$$

2.3.2. Determination of ash content

A porcelain crucible containing around 2 g of fish

Table 1. Hedonic characteristics and scores for organoleptic test

Attributes	Hedonic Characteristics	Hedonic choice	Hedonic score	Give tick (√) mark
Colour	Orangey Yellowish	Excellent	5	
	Light Orangey Yellowish	Very good	4	
	Brownish	Good	3	
	Whitish/gray	Low	2	
	Faint	Very low	1	
Odour	Very low fishy odour	Excellent	5	
	Low fishy odour	Very good	4	
	Normal fishy	Good	3	
	fishy odor	Low	2	
	Strong fishy odor	Very low	1	
Textures	Firm and flexible	Excellent	5	
	Slightly soft	Very good	4	
	Soft firm and flexible	Good	3	
	Soft	Low	2	
	Less firm and soft	Very low	1	
Broken Pieces	Nill	Excellent	5	
	Very low broken pieces	Very good	4	
	low broken pieces	Good	3	
	High broken pieces	Low	2	
	Very high broken pieces	Very low	1	

samples was placed in a muffle furnace for about six hours at about 550°C (AOAC, 2005). It was then chilled and weighed in desiccators. To verify ash completion, the crucible was again heated in a muffle furnace for 30 min, cooled, and weighed. This continued until the ash was

$$Ash\ content\ (\%) = \frac{Weight\ of\ the\ ash\ obtained}{Weight\ of\ the\ fish\ sample} \times 100\%$$

2.3.3. Estimation of protein content in fish by Micro Kjeldahal method.

The protein content of fish was measured using the standard methodology the Micro-kjeldahl technique (AOAC, 2005). The Micro-Kjeldahl method is based on the idea that when organic nitrogenous substances are digested with concentrated sulfuric acid in a suitable catalyst, most are entirely transformed into ammonium sulfate. Ammonia is liberated during steam distillation in a strong alkali presence and absorbed in the standard acid (boric acid). The total amount of nitrogen in the compound can be calculated from this amount of ammonia. Most proteins are composed of 16% nitrogen on average. In other words, one milliliter of nitrogen equals 6.25 milligrams of protein. Thus, the amount of protein can be determined from a known sample by determining the amount of nitrogen produced. The total nitrogen and protein were calculated using the following formula:

$$1000\ ml\ of\ 1N\ H2SO4 = 28\ gm\ of\ nitrogen$$

$$Z\ gm\ of\ nitrogen = 6.25 \times Z\ gm\ of\ protein$$

$$Protein\ (\%) = \frac{Burette\ reading \times gm\ of\ nitrogen \times 6.25 \times \frac{Total\ collected\ sample\ solution}{Volume\ of\ titrable\ sample\ solution}}{Weight\ of\ sample} \times 10$$

2.3.4. Estimation of lipid content

The lipid content of fish items measured by using Soxhlet device (model: FAT6, country of origin: Slovenia, manufacturer: India) and acetone extraction. (AOAC, 2005). The Soxhlet apparatus is built on a water bath using an iron stand and clamp. Then 5 g of the prepared sample was placed in a thimble filter in the Soxhlet apparatus. The sample was placed in an acetone-dipped, pre-weighed thimble. Pour an appropriate amount of acetone (50–100 ml) into the apparatus's circular joint flask. Heat the round joint flask gently in a water bath at a temperature appropriate for the solvent's boiling point (typically 700°C). When heated, the solvent evaporates, but it can descend gently after condensing on the sample inside the thimble. The solvent gradually builds up in the hollow space until the solvent containing fat drains out by siphoning into the round joint flask. The lipid process is repeated for 6 hours until all of the content in the sample has been extracted. Finally, the lipid-containing solvent is transferred to a pre-weighed beaker. The remaining lipid content is determined after the solvent has been removed by evaporating in a water bath.

After weighing the oil in the beaker with an electronic balance, the percentage of total lipid content was computed using the following formula:

$$\text{lipid content (\%)} = \frac{\text{Weight of lipid obtained}}{\text{Weight of fish sample}} \times 100$$

2.3.5. Determination of carbohydrate (CHO)

content

The proportion of carbohydrate content was estimated by subtracting the total percentages of protein, fat, moisture, and ash from 100. The CHO content of fish was calculated using the formula provided by (Ihekoronye & Ngoddy, 1985).

% of *CHO* content=100- {% moisture+% protein+% fat+% ash+% fiber}

2.4. Determination of nutrient loss during cooking

The nutrients are lost from the fish during frying and making curry. The lost weight is not only moisture but also some nutrients along with it. An electronic balance used to determine weight loss. The raw samples are weighed first, and after further processing, they must be weighed by balance. Then, we determined the loss of weight by deducting the last weight from the first weight. The following calculation was used to determine the percentage of weight loss provided by (Dryerre & Andross, 1946).

$$\text{Weight loss (\%)} = \frac{\text{Weight before cooking-weight after cooking}}{\text{Weight before cooking}} \times 100\%$$

2.4.1. Determination of total nutrient loss

The total nutrient loss (components other than moisture) can be determined by deducting the moisture difference between raw and cooked fish from the loss of weight. The following calculation was used to determine the percentage of weight loss provided by (Gerber et al., 2009).

Nutrient loss=Weight loss (%)-(moisture of raw sample-moisture of cooking sample)%

2.5. Peroxide value (POV) estimation

The procedure for determining the POV was presented by (Egron et al., 1981) and adopted from (Aurand & Woods et al., 1977). 1g of sample oil was precisely weighed into a stopper. To dissolve the lipid, a 250-ml conical flask was filled with 20 ml of chloroform. The flask was then shaken vigorously for 30 sec. A volume of 50 ml of a 3:2 combinations of acetic acid and chloroform was added. After adding one ml of saturated aqueous potassium iodide, the flask was spun for roughly 20 seconds before being placed in the dark for 30 min. Then, 100 ml of distilled water and 4-6 drops of starch indicator were added, and the released iodine was titrated against Na2S2O3.5H2O (0.002M). As an indication, a freshly made 1% starch solution was employed. The POV was calculated by the following formula:

$$\text{Peroxide value} = \frac{2\,(\text{sample titre - blank titre})}{\text{weight of sample oil}}\ \text{m.eq./kg of oil}$$

2.6. Determination of total volatile base nitrogen (TVBN)

Determination of total volatile base nitrogen was conducted using a method outlined in the Official Journal of the European Commission (E.C, 2002) and (AMC, 1979) with some specific adjustments.

TVBN values were calculated using prepared fish samples collected at time intervals. In a suitable container, 10 g of ground sample was weighed and combined with 90 ml of 6% perchloric acid. The samples were blended for 2 min in a cool environment (20–60°C). In a Kjeldahl flask, 100 ml of the extract was mixed with 4–6 drops of phenolphthalein. The flask was filled with 20 ml of a 20% NaOH solution and a few glass beads. The distillation outflow tube was immersed in a conical flask holding 50 mL of 3% boric acid solution and 1–2 drops of mixed indicator. Boil for 15-20 min in the flask. The distillate collected in the conical flask was titrated with 0.01N HCl, and the violet color of the mixed indicator confirmed the endpoint. The result is obtained from the following formula:

$$\text{Amount of TVB-N(mg / 100g sample)} = \frac{\text{ml titrant x 0.14}}{\text{Sample weight}} \times 100\%$$

2.7. Determination of heavy metals

First samples with 5g of boneless muscle tissue were extracted with the use of a stainless-steel knife and digested in strong acid digestion in a beaker with concentrated HNO_3 (65%) and H_2O_2 (35%) in a combination or at 1:3 proportions at 150°C for 20 min before chilling to room temperature followed by (Baharom & Ishak, 2015). Then it was diluted to 50 ml with ultra-pure water. Following filtering, the produced samples were tested for heavy metals such as Zn, Cu, Pb, Ni, Mn, and Cd using an atomic absorption spectrophotometer (AAS) and Inductively Coupled Plasma (ICM-MS). The standard element solution was diluting stock solutions of 100 mg/mL of each element from Perkin Elmer. Heavy metal concentrations were given in mg/kg, wet weight (ww) for fish and g/L for water. Finally, the results are compared to regulatory limits to assess the safety of the fish for consumption (Taweel et al., 2013).

2.8. Bacteriological and fungal experimentation

The bacteriological and fungal analysis was conducted following the method described by Harrigan (1998). A total of 100 ml of nutrient agar medium was prepared in a conical flask, thoroughly boiled to dissolve the ingredients, and then sterilized in an autoclave at 121 °C and 15 psi pressure for 30 minutes. The composition of the medium used for culturing bacteria and performing aerobic plate counts (APC) is summarized in Table 2. Specifically, 23.5 g of nutrient agar was suspended in 1 liter of distilled water and heated until completely dissolved. After sterilization, the medium was allowed to cool to approximately 50 °C, then poured into sterile Petri dishes under aseptic conditions.

Table 2. Composition of the bacterial cultural media

Ingredients	Quantity
Tryptone	5.0 gm/L
Yeast Extract	2.5 gm/L
Dextrose	1.0 gm/L
Agar	15.0 gm/L
Distilled water	1000 ml

To ensure uniform solidification, the media was poured at around 44 °C to coat the surface of pre-sterilized Petri dishes and allowed to cool and solidify. The aerobic plate count was conducted using the standard plate count method proposed by Seeley and Vandemark (1972), and results were expressed as colony-forming units (CFU/g). For sample preparation, each fish sample was first chopped using a sterile knife. Then, 25 g of chopped fish was transferred into a sterile blending jar, and 225 ml of 0.2% peptone water was added. The mixture was blended at 3000 rpm for two minutes, resulting in a 1:10 dilution. Using a sterile pipette, 1 ml of the blended solution was transferred into a test tube containing 9 ml of sterile 0.2% peptone water and thoroughly shaken. Serial ten-fold dilutions were then prepared as required, depending on the estimated bacterial load in each sample.

2.8.1. Standard plate count

The first 0.1 ml of a well-shaken, diluted sample was placed on nutrient agar plates in triplicate using a micropipette. The samples were transferred aseptically, and an L-shaped sterile glass spreader was used to evenly distribute the inoculum across the entire surface of the medium until it dried completely. The plates were then inverted and incubated at 30°C for 48 hours. After incubation, colonies were counted using a colony counter. Plates with more than 10 but fewer than 300 colonies were selected for enumeration, and results were expressed as colony-forming units per gram (CFU/g) of sample.

CFU = No.of visible bacterial colonies x dilution factor (CFU/gm or ml)

2.9. Statistical analysis

Descriptive statistics were as mean ± standard deviation using the statistical package SPSS v 26.0 (IBM Corp., Armonk, New York, NY, USA). Analysis of variance (One-way ANOVA) and Duncan's multiple range tests were used to determine the significance of differences among samples at a 95% confidence level ($p<0.05$).

3. Results and Discussion

3.1. Analysis of organoleptic characteristics of raw, curry and fried fishes

Table 3 showed that the findings of organoleptic characteristics of the three fish's (Ilish, Punti and Rui) raw, curry, and fried items based on color, odor, texture, and presence of broken parts. Every quality of each items showed significantly difference ($p<0.05$) among items. Current findings revealed that curry items retain maximum, excellent colour (5±0.00) and raw items retains minimum (2±0.00) colour. Strong fishy odour found in raw items (1±0.00) and very low odour found in curry items (5.00±0.0). Excellent texture found in raw items (5±0.00) and minimum texture in curry items (2.1±0.04). No significant difference in broken pieces, so all are excellent (5±0.00) except fried punti. In overall acceptable quality, curry items retain maximum (4.47±0.15) organoleptic quality and raw items retain minimum (3±0.00) organoleptic quality and significantly different ($p<0.05$) among each items, based on the scoring criteria of Table 1. This finding is closely related to the previous study described by Aberoumand et al. (2014), which demonstrated that the texture of fried and boiled fish fillets was significantly different; Majumdar et al. (2015) also said that cooking affects the color, flavor, and texture of the final product; and another author, Gaurat et al. (2020), very clearly showed that organoleptic features changed by various culinary techniques and that boiling fish was more acceptable than fried fish.

3.2. Analysis of proximate composition

The proximate composition of fish of the same species varies depending on age, sex, size, season of year, behaviors, and water status. The results of the proximate analysis of raw, curry, and fried items of fishes indicate some variations in their composition.

3.2.1. Moisture content analysis

The proximate composition of raw, curry, and fried samples of ilish, puti, and rui (three fish) is summarized in Table 4. Each fish shows a significantly different ($p<0.05$) value among each item. Raw fish items contain more moisture than curry, and curry fish items contain more moisture than fried items; these findings are similar to those of prior studies (Devadawson et al., 2019; Gaurat et al., 2020). The highest moisture content is found in raw items (74.13%), and the lowest moisture content is found in fried items (41.71%). According to previous studies, raw fish has (60–75%) (Van Ruth et al., 2014), (60.2–85.4%) (Bogard et al., 2015), or (68–81%) moisture (Ullah et al., 2022), curry fish contain 55.6% moisture (Majumdar et al., 2015), and fried fish contain 41.01% (Rukshana et al., 2021) and 43.12% (Abraha et al., 2018) moisture content. A little difference was shown between current and previous studies and could be attributed to differences in species, age, sex, size, growing region, season, cooking condition, etc. High-moisture-content fish are more susceptible to spoilage (Abbas et al., 2009). So, we can say that fried items retain the lowest moisture content and have a lower chance of spoilage (Zahid et al., 2011).

Data are the mean ± SD (n=10). Values in the same column with different superscripts (Ilish: a, b, c; Puti: m, n, o; Rui: x, y, z) are statistically significant from each other ($p < 0.05$) by Duncan Multiple Range Test (DMRT) of One-Way ANOVA.

Fish samples	Types of samples	Color	Odor	Texture	Broken pieces	Overall quality
Ilish	Raw	$2±0.00^{a}$	$1.02±0.1^{a}$	$5±0.00^{b}$	$5±0.00^{a}$	$3±0.00^{a}$
	Curry	$5±0.00^{c}$	$5.00±0.0^{c}$	$4.1±0.2^{a}$	$5±0.00^{a}$	$4.71±0.22^{c}$
	Fried	$3.02±0.16^{b}$	$4.23±0.3^{b}$	$5±0.00^{b}$	$5±0.00^{a}$	$3.62±0.13^{b}$
Puti	Raw	$2±0.00^{m}$	$2.97±0.11^{m}$	$5±0.00^{o}$	$5±0.00^{n}$	$3±0.00^{m}$
	Curry	$4.8±0.10^{o}$	$4.57±0.34^{o}$	$2.1±0.04^{n}$	$5±0.00^{n}$	$4.87±0.15^{o}$
	Fried	$3.01±0.15^{n}$	$3.93±0.2^{n}$	$1.37±0.25^{m}$	$4±0.00^{m}$	$4.01±0.06^{n}$
Rui	Raw	$2±0.00^{x}$	$1±0.00^{x}$	$5±0.00^{z}$	$5±0.00^{x}$	$3±0.00^{x}$
	Curry	$4.65±0.2^{z}$	$4.77±0.13^{z}$	$3.1±0.3^{x}$	$5±0.00^{x}$	$4.56±0.21^{z}$
	Fried	$3.96±0.2^{y}$	$3.23±0.1^{y}$	$4.63±0.17^{y}$	$5±0.00^{x}$	$4.1±0.12^{y}$

3.2.2. Ash content analysis

Table 4 showed the ash content of the three fish's raw, curry, and fried items. In these findings, we can see that the fried items contained more ash than the raw and curry items. These findings are closer to previous research (El-Lahamy et al., 2019). Fried items have significantly higher ash content ($p<0.05$) than raw and curry items, but raw and curry things have no significant difference. Fried items contain the highest (3.51%), and curry items retain the lowest (1.64%) ash content. According to previous research, raw fish contains 0.7–5.3% (Bogard et al., 2015), 3.26% (Abraha et al., 2018), curry fish contains 1.95±0.05% (Aberoumand et al., 2014), and fried fish contains 1.72–9.06% (Rahman et al., 2022), 5.39% (Abraha et al., 2018). Present findings are almost in the same range as prior findings, but this difference between the current and previous studies could be attributed to differences in species, age, sex, size, growing region, season, cooking condition, etc. Due to the moisture loss, the fried item has more ash. As a result, mineral composition and heavy metals such as cadmium, lead, mercury, etc. increased in fried items (Ersoy et al., 2006).

Table 4. Proximate composition of raw, curry and fried samples of ilish, puti and rui fish

Fish sample	Types of samples	Moisture (%)	Ash (%)	Protein (%)	Lipid (%)	Carbohydrate(%)
Ilish	Raw	55.2±0.62c	2.73±0.09a	19.62±0.37a	17.97±0.74a	4.49±1.27a
	Curry	50.97±0.3b	2.73±0.14a	20.3±0.48a	22.62±0.57b	3.39±1.24a
	Fried	41.71±0.64a	3.45±0.23b	23.94±0.3b	25.72±0.4c	5.13±1.34a
Puti	Raw	74±0.32o	1.77±0.14m	15.79±0.21m	5.41±0.53m	3.04±0.8mn
	Curry	67.35±0.44n	1.64±0.16m	18.82±0.35n	10.23±0.94n	1.98±0.27m
	Fried	50.4±0.92m	3.19±0.42n	24.36±0.76o	17.31±0.34o	4.75±0.43n
Rui	Raw	74.13±0.84z	2.67±0.14xy	15.01±0.31x	3.23±0.17x	4.99±1.06x
	Curry	70.16±0.31y	2.24±0.41x	18.28±0.23y	6.12±0.25y	3.22±0.44x
	Fried	56.34±0.89x	3.51±0.17y	21.89±0.38z	13.87±0.16z	4.41±0.34x
		56.34±0.89x				

*Values are mean ± standard deviation of three replicate. Values in the same column with different superscripts (Ilish: a, b, c; Puti: m, n, o; Rui: x, y, z) are statistically significant from each other ($p < 0.05$) by Duncan Multiple Range Test (DMRT) of One-Way ANOVA.

3.2.3. Protein content analysis

Table 4 showed that the protein content of the three fish's raw, curry, and fried items was significantly different ($p<0.05$) in all items of fish except raw and curry ilish. The protein content of three items decreased in the following order: fried items, curry items, and raw items, and this statement is on the right track according to previous studies (Bordin et al., 2013). The highest protein content is found in fried puti (24.36%), and the lowest protein content is found in raw rui (15.01%). According to previous studies, raw fish contains (11.9–20.6%) (Bogard et al., 2015), 52.6% (Abraha et al., 2018), curry fish contains (18–20%; 18–37%) (Puwastien et al., 1999; Ariño et al., 2013), and fried items contain (17–54%) (Puwastien et al., 1999) and 56.8% (Abraha et al., 2018) protein. The little difference between the current and previous studies could be attributed to differences in species, age, sex, size, growing region, season, cooking condition, etc. During frying, the fried samples lost more moisture than other fish samples, and as a result, nutrient concentrations also increased (Bordin et al., 2013). So, we can say that curry items and fried items of fish retain maximum protein.

3.2.4. Lipid content analysis

Table 4 shows that the lipid content of the three fish's raw, curry, and fried items is significantly different ($p<0.05$) among each item of fish. Fried items retain more lipid than other items in every fish; that statement is similar to a previous study (Moradi et al., 2011). Fried ilish retains the highest (25.72%), and raw rui retains the lowest (3.23%) lipid content. The fried items contained more lipids, while frying the fish with oil and curry items also had more lipids compared to raw. These occur because of weight loss during heating (El-Lahamy et al., 2019). According to the previous research, raw fish contains (0·6–14%) (Puwastien et al., 1999), 15.44% (Abraha et al., 2018) lipid, curry fish contains (0·5–15%), and fried fish contain (7–23%) (Puwastien et al., 1999), 21.23% (Abraha et al., 2018) lipid. The little difference between the current and previous studies could be attributed to differences in species, age, sex, size, growing region, season, cooking condition, etc. Some fish flesh tends to absorb more oil during cooking (Choe & Min, 2007). According to Puwastien et al. (1999), boiling and steaming did not affect the fat percentage in the cooked fish; however, roasting and frying increased the fat values of the cooked goods. So, we can say that fried items retain more lipid content than curry items.

3.2.5. Carbohydrate content analysis

The findings of this practical study in Table 4 on three fish items—raw, curry, and fried—are not significantly different ($p<0.05$) among each item of fish. Fried fish items contain more carbohydrates than other fish items. Fried ilish retains the highest (5.13%), and curry puti items retain the lowest (1.98%) carbohydrate content. According to previous studies, raw fish contains 2.31% (El-Lahamy et al., 2019), 3.67% (Chukwu et al., 2009) carbohydrate, curry fish contains 4.22% (Kolekar et al., 2012), and fried fish contains 0.68% (El-Lahamy et al., 2019) and 0.50% (Abraha et al., 2018) carbohydrate. The differences shown between the current and previous studies, especially in curry and fried items, could be attributed to differences in species, age, sex, size, growing region, season, cooking condition, etc.

3.3. Nutrition loss analysis

The findings of this study in Table 5 on curry and fried items of three fish show that fried items of every fish lose more nutrients than curry fish during cooking. Significant differences ($p<0.05$) were observed in each fish's curry and fried items. The highest nutrient loss occurs in fried items (18.41%), and the lowest nutrient loss occurs in curry items (10.75%). According to previous research, smoked fish lost approximately 5.3% (Kruijssen et al., 2020), roasted fish lost 38% (Farag et al., 2013), and fried fish lost 10.2% (Kruijssen et al., 2020) and 23% (Farag et al., 2013). The little difference present between the current and previous studies could be attributed to differences in species, age, sex, size, growing region, season, cooking condition, etc. Cooking temperature and time duration can significantly affect the weight loss of the fish items and also lead to increased nutrient losses (Uran & Gokoglu, 2014). From this study, we can clearly say that curry items of fish retain more nutrients than fried items of fish. So, curry fish is better for consumption.

Table 5. Nutrient loss (%) of curry and fried fishes

Type of fish	Types of sample	% of Nutrient Loss
Ilish	Curry	10.75 ± 0.48^{a}
	Fried	18.41 ± 0.74^{b}
Puti	Curry	11.31 ± 0.38^{m}
	Fried	17.24 ± 0.68^{n}
Rui	Curry	10.76 ± 0.37^{x}
	Fried	16.96 ± 0.85^{y}

*Values are mean ± standard deviation of three replicate. Values in the same column with different superscripts (Ilish: a, b; Puti: m, n; Rui: x, y) are statistically significant from each other ($p < 0.05$) by Duncan Multiple Range Test (DMRT) of One-Way ANOVA.

3.4. Total volatile base nitrogen (TVBN) analysis

TVBN content determination is one method used to determine the freshness of fish and fish products. Table 6 shows that the TVBN content of the three fish's raw, curry, and fried items is significantly different ($p<0.05$) among each item of fish. The highest value was found in fried items (14.03 mg/100 g) and the lowest value in raw items (6.61 mg/100 g). According to prior research, fresh fish contains 7.17 mg/100g (Majumdar et al., 2015), 10.46 mg/100g (Lakshman et al., 2015) of TVBN; boiled fish items contain 25 mg/100g (Farag et al., 2013); fried fish contains 13.12 mg/100g (El-Lahamy et al., 2019); (26.8–27.42) mg/100g (Lakshman et al., 2015); and 44 mg/100g

(Farag et al., 2013) of TVBN. Castro et al. (2012 reported that up to 25 mg/100g of TVBN in fish is considered high quality, up to 30 mg/100g is good quality, the limit of acceptability is up to 35 mg/100 g, and fish are spoiled when above 35 mg/100g (Ludorff & Meyer, 1973). The little difference present between the current and previous studies could be attributed to differences in species, age, sex, size, growing region, season, cooking condition (temperature, time), etc. From the above discussion, we can say that the present study findings do not cross the limit of acceptability, and curry fish is more preferable than fried fish.

3.5. Per-oxide value (POV) analysis

Table 6 shows that the per-oxide value of the three fish's raw, curry, and fried items is significantly different ($p<0.05$) among each item of fish. From the findings, we can see that fried fish contain a higher POV than curry, and curry items contain a higher POV than raw fish. The highest value was found in fried ilish (65.54 m.eq./kg of oil), and the lowest value was in raw rui (6.43 m.eq./kg of oil). According to Low et al. (1992), POV values lower than 10 meq/kg are considered fresh, and in the range of 20 to 40 meq/kg of oil, rancid taste begins to be apparent. Another study said the POV value of fresh fish oil should be 5 to 10 meq/kg of oil (Kesbiç et al., 2023). According to the acceptance rate of previous research and the present study findings, we can clearly see that the maximum findings are beyond the acceptance rate. This difference could be attributed to differences in species, age, sex, size, growing region, season, cooking condition (temperature, time), lack of storage, etc. Curry items retain less POV than fried items, so curry items are more preferable.

Fish sample	Types of samples	POV (m.eq./kg of oil)	TVBN (mg /100gm)
Ilish	Raw	42.45±0.58^{a}	7.91±0.4^{a}
	Curry	51.31±0.44^{b}	11.88±0.54^{b}
	Fried	65.54±0.47^{c}	13.9±0.49^{c}
Puti	Raw	15.29±0.45^{m}	6.89±0.29^{m}
	Curry	24.69±0.23^{n}	8.99±0.34^{n}
	Fried	42.16±0.37^{o}	13.22±0.47^{o}
Rui	Raw	6.43±0.28^{x}	6.61±0.58^{x}
	Curry	17.78±0.33^{y}	10.9±0.4^{y}
	Fried	35.74±0.18^{z}	14.03±0.27^{z}

*Values are mean ± standard deviation of three replicate. Values in the same column with different superscripts (Ilish: a, b, c; Puti: m, n, o; Rui: x, y, z) are statistically significant from each other ($p < 0.05$) by Duncan Multiple Range Test (DMRT) of One-Way ANOVA.

3.6. Heavy metals composition in fish

In the present study, Table 7 demonstrated that the heavy metal composition of the three fish's raw and curry items is significantly different ($p<0.05$) among each item of fish. WHO/FAO recommended the maximum permissible limit of heavy metals also presented in Table 7.

Table 7. Heavy metals composition of raw, curry, and fried samples of ilish, puti, and rui fish also WHO and FAO, compilation of legal limits for hazardous substances in fish and fishery (Nauen, 1983; and FAO, 1989)

Type of fish	Types of samples	As (mg/kg)	Cd (mg/kg)	Cr (mg/kg)	Pb (mg/kg)	Ni (mg/kg)	Zn (mg/kg)
Ilish	Raw	2.04±0.13^{a}	0.15±0.02^{a}	0.37±0.03^{a}	0.35±0.07^{a}	1.67±0.3^{a}	12.47±1.17^{a}
	Curry	2.67±0.25^{b}	0.18±0.02^{a}	0.46±o.o4^{b}	0.29±0.007^{a}	1.74±0.07^{a}	11±0.35^{a}
Puti	Raw	2.07±0.27^{m}	0.6	ND	1.87±0.24^{m}	1.4±0.2^{m}	8.4±0.2^{m}
	Curry	2.74±0.07^{n}	ND	ND	2.63±0.14^{n}	1.87±0.07^{n}	9.54±0.25^{n}
Rui	Raw	4±0.31^{x}	0.18±0.02^{x}	2±0.12^{x}	1.84±0.15^{x}	2.3±0.44^{x}	16.34±1.21^{x}
	Curry	4.37±0.22^{x}	0.26±0.03^{y}	2.14±0.07^{x}	2.74±0.39^{y}	3±0.15^{x}	17±0.58^{x}
WHO		1	1	1	2	1	100
FAO		1	2	1	1-5	1	100

*Values are mean ± standard deviation of three replicate. Values in the same column with different superscripts (Ilish: a, b, c; Puti: m, n, o; Rui: x, y, z) are statistically significant from each other ($p < 0.05$) by Duncan Multiple Range Test (DMRT) of One-Way ANOVA. ND=Not detected.

Among the findings of heavy metals, zinc is present in the highest amount (17±0.58) mg/kg in curry items and the lowest amount (8.4±0.2) mg/kg in raw items. According to previous research in Bangladesh, raw fish contains 6 to 47 mg/kg (Bogard et al., 2015), 35.6 ± 5.71 mg/kg (Islam et al., 2020), and boiled items contain 39.0 ± 0.35 mg/kg (Islam et al., 2020). The maximum acceptable limit of Zn in fish and fish products was 100 mg/kg, as recommended by FAO, WHO, and the European Union (30 mg/kg) (Sarker et al., 2020). The study suggested that the zinc content of fish is not hazardous for human consumption.

In arsenic (As), the highest value is found in curry items (4.37±0.22) mg/kg and the lowest value in raw items (2.04±0.13) mg/kg. This inconvenience might be caused by reducing the overall mass of the sample but does not remove heavy metals like arsenic, which are not destroyed by heat. According to prior research in Bangladesh, raw fish contains 3.55 mg/kg (M. S. Rahman et al., 2012), 0.89 mg/kg (Sarker et al., 2020), and boiled and fried fish contain 0.6 and 10 mg/kg, respectively (Islam et al., 2020). The maximum acceptable limit of As was 1 mg/kg, as proposed by FAO (1983) and WHO (1989), and the acceptable limit is 5 mg/kg in Bangladesh (Sarker et al., 2020). The present findings showed that all items of fish acceptable range by the national acceptable limit of As. Long-term exposure to arsenic has been linked to cancer in the skin, kidney, lung, bladder, and other organs. Arsenic is known to be a human carcinogen (M. Alam et al., 2023).

In nickel (Ni), the highest value is found in curry items (3±0.15) mg/kg and the lowest value in raw items (1.4±0.2) mg/kg. According to prior research in Bangladesh, raw fish contains (0.04–21.4) (Islam et al., 2015), 2.59 mg/kg (Rahman et al., 2012), and 0.06 mg/kg (Parvin et al., 2023) and the nickel content of processed fish's has been reduced from fresh fish (Öztürk et al., 2009). The maximum safe limit of Ni was 1 mg/kg, as recommended by FAO (1983) and WHO (1989). The present findings showed that all items of fish exceed the acceptable limit of Ni. Ni poisoning has several detrimental effects, such as skin irritation and damage to the lungs and nervous system (Alam et al., 2023).

In chromium (Cr), the highest value is found in curry items (2.14±0.07) mg/kg and the lowest in raw items (0.37±0.03 mg/kg), but chromium is not present in items of puti fish. According to previous studies, raw fish contains 1.31 mg/kg (Sarker et al., 2020), 1.12 mg/kg (Rahman et al., 2012), and 0.23 mg/kg (Parvin et al., 2023), and boiled fish muscle contains 4.8 mg/kg (M. A. Islam et al., 2020). The maximum acceptable range of chromium is 0.1–1.0 mg/kg, as established by FAO, 1983 and WHO, 1989 (Sarker et al., 2020). In the present findings, after cooking one curry item, it exceeds the acceptable limit. A higher amount of Cr in the diet has a serious effect on lipid and glucose metabolism and is also responsible for kidney, lung, and liver disease (Alam et al., 2023).

In lead (Pb), the highest value was found in curry items (2.74±0.39) mg/kg and the lowest value in curry items (0.29±0.007) mg/kg. According to prior research in Bangladesh, raw fish contains 0.22 mg/kg (Parvin et al., 2023), 5.0 mg/kg (Islam et al., 2020), (0.04–1.6) mg/kg (Islam et al., 2015), and boiled samples contain 6.2 mg/kg (Islam et al., 2020) of Pd. The permissible limits of Pd are 1–5 mg/kg (FAO, 1983), 2 mg/kg (WHO, 1989), up to 3 mg/kg (JECFA), and 0.3 mg/kg in Bangladesh (Sarker et al., 2020). The present findings stayed within the safe limit, according to FAO. Excess Pb intake through diet may cause nephrotoxicity, neurotoxicity, etc. health problems (Alam et al., 2023).

In cadmium (Cd), the highest value found in curry items is 0.6 mg/kg, and the lowest value is 0.15±0.02 mg/kg, but Cd is not present in curry puti items. According to prior research in Bangladesh, fresh fish retains 0.01 mg/kg (Parvin et al., 2023), (0.04–1.6) mg/kg (Islam et al., 2015), and 0.09–0.87 mg/kg (Rahman et al., 2012) on a dry basis, and Huque et al. (2014 showed that there were no significant differences between fresh and boiled fish. The permissible limits of Cd are 0.1 mg/kg (FAO, 1983; WHO, 1989) and in Bangladesh, 0.25 mg/kg (Sarker et al., 2020). The maximum findings are slightly higher than the WHO/FAO acceptable limit. Renal failure, weakened bones, and prostate cancer can all be consequences of high-dose or prolonged exposure to cadmium pollution (Alam et al., 2023).

It is almost similar to the prior study, but there is little difference between the current and previous studies, which could be attributed to differences in species, age, sex, size, growing region, season, cooking conditions, etc. From the above discussion, we can clearly say that As, Ni, and Cr are present in higher amounts in these raw and curry items of fish, and Cr exceeds the limit in only rui fish, but Zn and Cd are present in the range of permissible limits. Another decision from these findings is that curry items contain more heavy metals than raw fish.

3.7. Bacteriological and fungal analysis

Table 8. Standard Plate Count (SPC) for bacteria and fungus of raw, curry and fried fishes

Fish sample	Types of samples	Bacteria (CFU/g)	Fungi (CFU/g)
Ilish	Raw	$3.5 \pm 0.61 \times 10^{4\,b}$	$3.08 \pm 0.54 \times 10^{5\,c}$
	Curry	166.67 ± 29.6^{a}	$4.4 \pm 0.42 \times 10^{2\,a}$
	Fried	100 ± 15.28^{a}	$1.6 \pm 0.33 \times 10^{3\,b}$
Puti	Raw	$2.26 \pm 0.25 \times 10^{4\,n}$	$6.89 \pm 0.29 \times 10^{5\,o}$
	Curry	183.34 ± 14.52^{m}	$8.99 \pm 0.34 \times 10^{2\,m}$
	Fried	130 ± 15.28^{m}	$13.22 \pm 0.47 \times 103^{n}$
Rui	Raw	$2.1 \pm 0.18 \times 10^{4\,y}$	$4 \pm 2 \times 10^{5\,z}$
	Curry	266.67 ± 29.63^{x}	$4.79 \pm 0.18 \times 10^{2\,x}$
	Fried	200 ± 11.55^{x}	$2.14 \pm 0.14 \times 10^{3\,y}$

*Values are mean ± standard deviation of three replicate. Values in the same column with different superscripts (Ilish: a, b, c; Puti: m, n, o; Rui: x, y, z) are statistically significant from each other ($p < 0.05$) by Duncan Multiple Range Test (DMRT) of One-Way ANOVA.

Table 8 shows the bacteriological analysis of the three fish's raw, curry, and fried items. Raw fish showed significantly ($p<0.05$) higher amount of bacteria compare with curry and fried items, but there was no significant difference stayed between curry and fried. The highest colony-forming unit of bacteria ($3.5\pm0.61\times10^4$ CFU/g) was found in raw items, and the lowest value (100 ± 15.28 CFU/g) stayed in fried items. This finding in fried item might be reasoned by frying temperature. According to previous research, raw fish retains 1.84×10^6 CFU/ml (Dutta et al., 2018), 2.774 $\times10^6$–4.416×10^6 (Udochukwu et al., 2016; Eizenberga et al., 2015), 5.2×10^4 CFU/g (Nur et al., 2020), 6.13×10^5 cfu/g (Yohans et al., 2022), and cooked fish items (both curry and fried items) hold 2.81×10^4 cfu/g (Yohans et al., 2022) bacteria. The little difference present between the current and previous studies could be attributed to differences in species, growing region, cooking conditions (temperature, time), etc. So, we can say that raw fish might be a concern for public health.

On the other hand, Table 6 also showed that the fungal analysis of the three fish's raw, curry, and fried items was significantly different ($p<0.05$) among each item of fish. The highest fungus colony was found in raw items ($6.89\pm0.29\times10^5$ CFU/g), and the lowest was in curry items ($4.4\pm0.42\times10^2$ CFU/g). This difference in result might be reasoned cooking temperature. According to previous research, raw fish contains 5.787×105–1.840×10^6 cfu/g (Udochukwu et al., 2016), up to 106 CFU/g (Nur et al., 2020), 1.8×104–7.0 × 10^4 cfu/g (Dutta et al., 2018), cooked items contain 5.6×101 cfu/g (Mitiku et al., 2023), and fried items contain 3.72×10^2 (Odhiambo et al., 2018) fungus. Similar to prior studies, the slight differences between the current and previous studies could be attributed to variations in species, growing region, cooking conditions (temperature, time), and other factors. So, we can clearly say that curry items were more preferable for consumption than raw and fried fish.

Our research has several limitations. Firstly, we used only three fish species, which is a relatively small number compared to the large number of fish species. We did not account for the heavy metal analysis of fried fish and several crucial factors, including mercury (Hg) and various vitamins. Further study is recommended to find the shortcomings of the study.

4. Conclusion

The current study examined the raw, curry, and fried items from three different fish species. It concluded that raw fish was more susceptible to spoilage due to its higher microbial load, posing a serious concern for public health. In curry items, fish retain the best organoleptic characteristics, minimal nutrient loss, and a minimum microbial effect compared to raw and fried items. Fried fish items showed minimum moisture content, but maximum in protein, ash, lipid, and carbohydrate contents, and significantly higher nutrient loss and microbial effects than raw and curry items. Between raw and curry items, curry items retain a higher amount of heavy metals than raw fish, and Ni exceeds the acceptable limits in both items. The amounts of Pb, Cd, and Zn were present at a safe level according to the WHO/FAO maximum safety limit. Mercury (Hg) analysis and heavy metals in fried fish are recommended for further study. By bio-chemical analysis, the maximum peroxide and maximum total volatile base of nitrogen present in fried items, which might be of concern to public health. Furthermore, we can say that fried items were highly affected by culinary technique rather than other items, which potentially increases the risk of cancer. Overall, this investigation revealed that curry items are the best way to preserve the nutritional value of fish.

Ethical statement

Not applicable.

Declaration of competing interest

No conflicts of interest have been disclosed by the authors.

Data availability

Data will be made available on request.

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