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Nutrient and heavy metals composition of dried fish varieties from Bangladesh

Huan Sun[a], Derek S. Johnson[b], Rotimi E. Aluko[a,c,*]

[a] Department of Food and Human Nutritional Sciences, University of Manitoba, Winnipeg, MB, R3T 2N2, Canada
[b] Department of Anthropology, University of Manitoba, Winnipeg, MB, R3T 2N2, Canada
[c] Richardson Centre for Food Technology and Research, University of Manitoba, Winnipeg, MB, R3T 2N2, Canada

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ABSTRACT

This work determined the nutritional value of some dried fishes that are commonly consumed in Bangladesh. Protein content was significantly ($p < 0.05$) higher in the white sardine (72.5 g/100 g) and Ganges River sprat (74.2 g/100 g) with essential amino acids score of 137.7% and 136.3%, respectively. The fermented anchovies had significantly ($p < 0.05$) higher free amino acids content (4.4 g/100 g) when compared to the other dried fishes with 0.7–2.5 g/100 g values. However, the ribbon fish had significantly ($p < 0.05$) higher protein digestibility of 84.0% when compared to <80% for the other fishes. Significantly ($p < 0.05$) lower sodium but higher potassium contents were found in white sardine and Ganges River sprat. White sardine also had the highest contents of eicosapentaenoic acid (6.5 g/100 g of oil), docosahexaenoic acid (19.5 g/100 g of oil), and total n-3 fatty acids (27.6 g/100 g of oil). The freshwater barb (fermented or unfermented) contained the highest contents of monounsaturated fatty acids and lowest levels of saturated fatty acids ($p < 0.05$). The heavy metals contents in white sardine were less than the maximum permitted levels. However, Ganges River sprat had significantly higher cholesterol content (12.5 mg/g), which may limit its nutritional value.

1. Introduction

The global fishery and aquatic production levels reached a record high of 122.6 million tons in 2020 (FAO, 2022). As per capita consumption of aquatic foods continues to rise, the Food and Agriculture Organization of the United Nations (FAO) emphasizes that the fishery sector continually plays a crucial role in addressing the needs of population growth-related food stress and nutritional requirements (FAO, 2022). Asia accounts for 70% of the world's total fishery production, where the leading producers namely, China, Indonesia, India, Vietnam, Japan, and Bangladesh are located (FAO, 2022). Notably, Bangladesh's fishery industry is unique due to its heavy reliance on inland aquaculture, which constitutes 56.76% of the country's total production (Hasan et al., 2021). In facing the world food crisis, the FAO underscores the importance of sustainability in fisheries to alleviate hunger and nourish people worldwide (FAO, 2022). Therefore, research on the quality of fishery products is essential for promoting fishery sustainability and food security.

Malnutrition is widespread worldwide, particularly affecting newborns, children, and adolescents, making them more susceptible to diseases (Black et al., 2013). In Bangladesh, 36% of children under the age of 5 years are stunted, and the wasting rate of 14% is among the highest in the world, both of which are rooted in malnutrition (NIPORT & Macro, 2005; WHO & UNICEF, 2017). Micronutrients deficiencies, including vitamin B_{12}, iron, zinc, calcium, and n-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA), affect 2 billion individuals worldwide and are exacerbated by poor dietary diversity (FAO, 2013; Nordhagen et al., 2020). The impoverished population in Bangladesh faces serious malnutrition challenges due to a lack of high-quality dietary proteins from animal and plant sources (Rahman et al., 2012). The prevalence of iron deficiency anemia in pregnant women and children is a special public health challenge in Bangladesh (ICDDRB, 2013).

As one of the main forms of stored fish, dried fish (DF) has a long history of production and consumption in the world, especially in developing countries. Sun-dried fishes are considered nutrient-rich sources of proteins, lipids, minerals, and vitamins (Bhowmik et al., 2022). In Bangladesh, it has been reported that Bengal DFs are rich in protein (more than 50%, dry weight basis), calcium, iron, zinc and n-3 LC-PUFA, which contribute to enhanced human health, especially through the contents of eicosapentaenoic acid (EPA) and

* Corresponding author. Department of Food and Human Nutritional Sciences, University of Manitoba, Winnipeg, MB, R3T 2N2, Canada.
E-mail address: rotimi.aluko@umanitoba.ca (R.E. Aluko).

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docosahexaenoic acid (DHA) (Bhowmik et al., 2022). Furthermore, the cost-effectiveness of DFs offers a cheaper price per unit of protein in comparison to fresh fish. Therefore, consumption of DFs is considered as a suitable dietary practice to alleviate public health pressure rooted in the prevalence of malnutrition among vulnerable groups (Hossain et al., 2017).

Despite existing studies that have contributed to understanding the nutrient composition of DFs, gaps still exist in the field as most of the studies focused on sampling in specific areas and the reported data relates mainly to proximate composition. Therefore, there is a need for more comprehensive and systematic studies of the nutritional value of DFs. In this study, seven DFs collected from different markets in Bangladesh were evaluated for nutritional quality using proximate composition as well as contents of minerals, heavy metals, amino acids, fatty acids, vitamin B_{12}, and cholesterol. Estimation of the potential contribution of regular consumption of DFs to the overall nutritional health of specific population groups was also determined for some of the nutrients.

2. Materials and methods

2.1. Raw materials and sample preparation

The following types of sun-dried DF were purchased from local markets in Dhaka, Bangladesh, and then transported to the laboratory: Bombay duck (BD, *Harpadon nehereus*); white sardine (WS, *Escualosa thoracata*); Ribbon fish (RF, *Trichiurus lepturus*); Freshwater barb (FB, *Puntius* spp.); Fermented barb (FM, *Puntius* spp.); Fermented anchovies (FA, *Setipnna* spp.); and Ganges river sprat (GR, *Corica soborna*). Evisceration was performed on BD and RF, while the other fish samples underwent minimal processing prior to sun-drying and salting. Each fish type was purchased from 3 different popular markets and analyzed individually to obtain samples that represent consumer purchases. Upon arrival, the samples were stored at −20 °C until used for the experiments.

2.2. Chemical reagents

Chemical reagents used in the study were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Fisher Scientific Company (Oakville, ON, Canada). All the chemicals and reagents were of analytical grade. The DFs were thawed at 4 °C overnight, oven dried (Isotemp oven 516, Fisher Scientific, CA) at 50 °C for 12 h, then grounded into a fine powder using a coffee grinder, and stored in airtight containers at −20 °C.

2.3. Proximate and mineral composition analysis

Moisture, protein, ash, fibre, and minerals of the fish powders were determined according to the relevant Association of Official Analytical Chemists' methods (Horwitz, 1997). Fat content determination was followed the American Oil Chemists' Society methods (Mehlenbacher et al., 2009).

2.4. Heavy metals

Mercury (Hg) content was determined by US EPA method 7473, conducted on a Hydra IIC (Teledyne Technologies, Thousand Oaks, CA, USA). The contents of chromium, cadmium, lead, and arsenic were determined by the method outlined in the Association of Official Analytical Chemists' methods 2015.01 (Briscoe, 2015).

2.5. Amino acid composition

Amino acid profiles of the DFs were determined using the HPLC Pico-Tag system according to the method previously described (Bidlingmeyer et al., 1984). The cysteine and methionine contents were determined after performic acid oxidation (Gehrke et al., 1985), and the tryptophan after alkaline hydrolysis (Landry & Delhaye, 1992).

The digestible essential amino acid score (DEAAS) of a sample can be summarized as the minimum contribution of one or multiple essential amino acids (mg/g of protein) to the reference quantity for >18 years old (FAO Expert Consultation, 2011). The contribution corresponding to each essential amino acid in the sample was calculated, and the value of the amino acid with the lowest percentage contribution adopted as the DEAAS of the sample. The formula is as follows (FAO Expert Consultation, 2011):

DEAAS (%) = min [percentage contribution of histidine, leucine, isoleucine, lysine, threonine, tryptophan, valine, sulfur containing amino acid, aromatic amino acids]   (1)

2.6. Muscle protein composition

The extraction of sarcoplasmic and myofibrillar proteins was performed using the method described by Hashimoto et al. (1979).

In brief, 2.5 g of fish powder was homogenized with 25 mL of phosphate buffer (pH 7.5) to prepare suspension I. After centrifugation of suspension I (5000×*g*, 15 min), supernatant I and precipitate I were obtained. Precipitate I was then homogenized with an additional 25 mL of phosphate buffer (pH 7.5), resulting in suspension II. Following centrifugation of suspension II (5000×*g*, 15 min), supernatant II and precipitate II were collected. Supernatant I and supernatant II were combined, and trichloroacetic acid (TCA) was added to a final concentration of 5 g/100 mL. The TCA mixture was centrifuged (5000×*g*, 15 min), and the precipitate collected and freeze-dried as the sarcoplasmic protein.

Precipitate II was homogenized with 25 mL of KCl-phosphate buffer (pH 7.5) to create suspension III, which was then centrifuged (5000×*g*, 15 min) to obtain supernatant III and precipitate III. Precipitate III was subsequently homogenized with another 25 mL of KCl-phosphate buffer (pH 7.5), followed by centrifugation (5000×*g*, 15 min) to collect supernatant IV. Supernatants III and IV were combined and freeze-dried to obtain the myofibrillar protein. The protein content of each fraction was determined by the modified Lowry method (Markwell et al., 1978).

2.7. Free amino acids

The free amino acids extraction followed the method of Goto et al. (2021) while identification of individual free amino acids was carried out using the Pico-Tag method (Bidlingmeyer et al., 1984).

2.8. In vitro protein digestibility

The *in vitro* digestibility was determined using previously outlined protocols (Hsu et al., 1977) with some modifications. Thirty milliliters of an aqueous DF mixture (6.25 mg protein/mL) was adjusted to pH 8 with 0.1 mol/L NaOH while stirring at 37 °C. One milliliter of an enzyme mixture (4.8 mg/mL trypsin at 1–2 mkat, 9.3 mg/mL chymotrypsin at ≥0.67 μkat, and 3.9 mg/mL peptidase at 0.58 μkat) was then added to the DF mixture. The drop in pH of the mixture was recorded at 30 s intervals over a 10 min period using a pH meter. The percentage protein digestibility of each DF was determined as follows using the regression equation of Hsu et al. (1977).

$$\%\ Protein\ digetibility\ (Y) = 210.46 - 18.10Xf \quad (2)$$

where *Xf* is the final pH value of the mixture after a 10 min digestion.

2.9. Cholesterol content

Cholesterol was determined using the protocol outlined by Van

Elswyk et al. (1991) with some modifications. Briefly, 200 mg of DF powder was mixed with 1.6 mL of 50 g/100 mL KOH and 8 mL of 95 mL/100 mL ethanol in a capped glass centrifuge tube and incubated in a 40 °C water bath for 1 h. The mixture was cooled to room temperature, and vortexed with 8 mL of water and 12 mL hexane for 1 min. The upper layer of hexane was collected after centrifuging the mixture at 1100×*g* for 12 min. The aqueous layer was re-extracted thrice consecutively with 12 mL of hexane. The hexane layers were combined and washed five times by adding 10 mL of distilled water. The hexane layer was further extracted with 500 mg of Na_2SO_4 for 15 min and a 1.5 mL aliquot evaporated with nitrogen flush in a 40 °C water bath. One mL of cholesterol standard (2 mg/mL in ethanol) was prepared the same way as the DF and 0, 45, 90, 180, 900, 1800 and 3600 μL aliquots evaporated with nitrogen flush in the 40 °C water bath. Two mL of freshly prepared o-phthalaldehyde (0.5 mg/mL in glacial acetic acid) were thoroughly mixed and incubated with the evaporated cholesterol extract for 10 min in the dark. One mL of concentrated sulfuric acid was added into the mixture, then thoroughly mixed and incubated in the dark for another 10 min. Absorbances vs. concentrations of the standard solutions at 550 nm were used to obtain a linear regression equation. The cholesterol content of DF samples was calculated using the regression equation obtained from the plot of absorbance versus concentration of the standard solutions.

2.10. Vitamin B_{12} content

The vitamin B_{12} content was determined by the method of analysis for infant formulas using the Association of Official Analytical Chemists' methods 952.20 and 960.46 (Deutsch, 1994).

2.11. Fatty acids composition

The fish lipids were extracted following a standard protocol (Folch et al., 1957). Briefly, 1 g of DF powder was mixed with 4 mL of 0.025 mol/L $CaCl_2$ solution and 20 mL of chloroform:methanol (2:1, v/v) solvent in a 50 mL glass centrifuge tube, and then vortexed for 2 min. The bottom phase was collected, centrifuged (500×*g* for 12 min), and evaporated in a new 12 mL screw-capped glass tube using a nitrogen evaporator at 40 °C in a water bath. The extracted lipids were subjected to boron trifluoride (BF_3)-catalyzed methylation. Briefly, 1.5 mL of methanolic BF_3 solution (14 mL/100 mL) and 2 mL of hexane were added into the lipid-containing 12 mL screw-capped glass tube and heated for 1 h and 30 min at 108 °C. After cooling the tube to room temperature, 1 mL of distilled water was added into the tube and vortexed for 20 s. The mixture was subsequently centrifuged at 500×*g* for 12 min, and the upper phase collected into a pre-weighed 12 mL glass tube (W_1) followed by evaporation with nitrogen at 40 °C in a water bath. The weight of the tube with lipids was recorded as W_2 and the total weight of the lipids was calculated as:

$$Total\ lipd\ weight = W_2 - W1 \quad (3)$$

A 10 mg/mL of lipid solution was prepared by diluting the methylated lipids in hexane and then injected into a Bruker 450-GC gas chromatography system (Bruker, Billerica, MA, USA). The GC included a flame ionization detector and a DB225MS column (30 m × 0.25 mm; Agilent Technologies Canada Inc., Ontario, Canada). The fatty acids were identified on the chromatogram by conventional methods using the retention time of standards.

2.12. Statistical analysis

Each fish sample was analyzed in duplicate and results from the three markets were used to calculate the mean value for the DF type. Data was analyzed through one-way ANOVA using IBM SPSS 28.0.0.0 (IBM, Armonk, NY, USA) and reported as mean ± standard deviation. The statistical differences were determined by Duncan's multiple range test ($p < 0.05$).

3. Results and discussion

3.1. Proximate composition

The proximate composition of DFs is critical as it provides an initial indication of the food quality standards of DFs as required for food regulations (Ahmed et al., 2022). Proximate composition of the DFs is shown in Fig. 1. The moisture contents were under 13%, aligning with the values reported by Bhowmik et al. (2022). Haider et al. (2021) suggested that moisture contents below 20% are essential for maintaining quality and ensuring long-term preservation of DFs because microbial growth is inhibited. Therefore, the samples used in this study could be considered to have the prerequisite moisture levels needed for long-term shelf life.

The protein contents of the DFs ranged from 46 g/100 g–74 g/100 g, which are similar to the 51 g/100 g–77 g/100 g values reported by Kar et al. (2020). The higher protein contents of WS and GR make them better protein-rich food materials than the other DFs. Fish protein is considered complete as it contains all the essential amino acids required for the normal functioning of major organs such as brain, hearts, and eyes (Hei, 2018). In addition, according to Vikøren et al. (2013), short-term daily supplementation with low doses of fish protein may benefit through reduced blood glucose and LDL cholesterol levels as well as improved glucose tolerance in overweight adults. Another study reported that β-parvalbumin in fish protein may have the potential to inhibit neurodegenerative diseases such as in Alzheimer's and Parkinson's by inhibiting the formation of amyloid structures (Werner et al., 2018).

The FB and FM with the highest fat contents ($p < 0.05$) were found to be made from *Puntius* spp. In a recent study (Bhowmik et al., 2022), it was found that the fat content of Punti powder developed from *Puntius ticto* whole fish was 17.58 g/100 g, which is consistent with the current results. Rana et al. (2020) also reported that Puti, a DF developed from *Puntius sophore*, had a higher fat content when compared with Kachiki (GR), Churi (RF), and Loita (BD). According to Ahmed et al. (2022), the relationship between moisture content and fat content in fresh fish is negatively correlated. Interestingly, a similar correlation was also observed for DFs in the current study. This trend is particularly evident in the data showing *Puntius* spp. DFs with low fat contents but high moisture contents, while the opposite is the case for *Trichiurus lepturus*.

In general, the ash content comes mainly from the viscera, bones and fins (Bhowmik et al., 2022). Therefore, factors such as fish species can be a main factor that determines the ash content. In the current study, FB and FM made from *Puntius* spp. and FA made from *Setipnna* spp. were found to have higher ash contents ($p < 0.05$), which are consistent with the results reported by previous studies (Kar et al., 2020; Rana et al., 2020). Given that salt is used as a preservative in DFs production and storage (Paul et al., 2018), the high ash contents observed in FA, FB, and FM may be attributed to the excessive addition of salt. Fibre is not a major component of fishes and as shown in Fig. 1, the levels are mostly insignificant (<0.3 g/100 g) in comparison to the other nutrients.

3.2. Mineral composition

The mineral composition of the DFs is summarized in Table 1, which consists of the macro-minerals (phosphorus, potassium, calcium, sodium, and magnesium) presented as weight percentage (g/100 g) and micro-minerals (manganese, iron, copper, and zinc) presented as mg/kg.

Sodium is one of the most abundant elements found in the DFs, among which FB and FM made from *Puntius* spp. had the highest levels. These findings are consistent with the report of Bhowmik et al. (2022), that the whole Punti powder developed from *Puntius ticto* contained more sodium when compared with whole Kachki (GR) powder. An appropriate amount of dietary sodium helps to maintain the normal

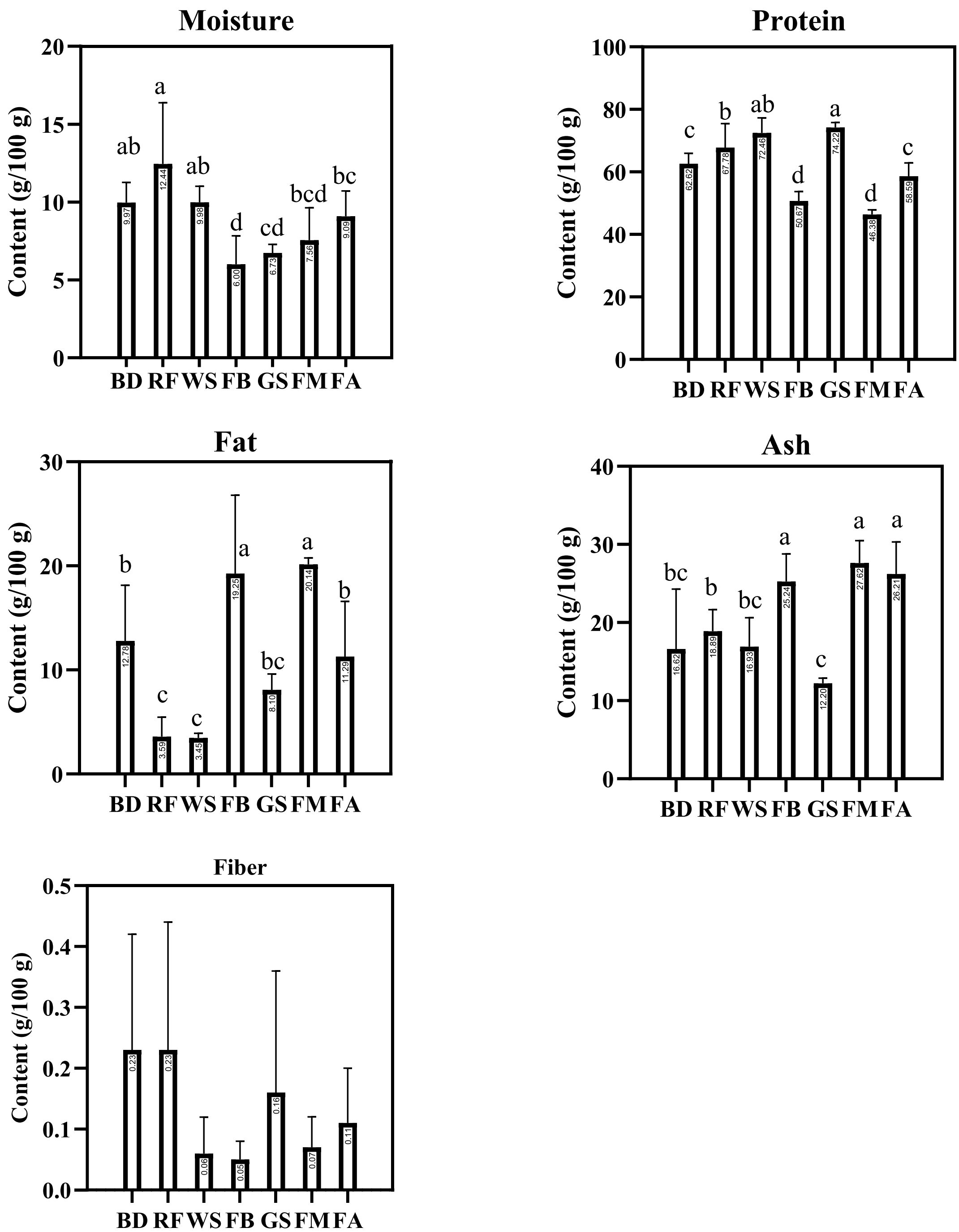

Fig. 1. Proximate composition (g/100 g) of dried fishes obtained from Bangladesh. BD, Bombay duck; RF, Ribbon fish; WS, White sardine; FB, Freshwater barb; GS, Ganges River sprat; FM, Fermented barb; FA, Fermented anchovies. For each graph, bars with different letters have significantly ($p < 0.05$) different mean values.

extracellular fluid composition, as well as balance the water, acid-base, and salt levels in the body (Quintaes & Diez-Garcia, 2015). However, high sodium intake is known to be related to the onset of hypertension, cardiovascular diseases, and chronic kidney disease (O'Donnell et al., 2020). According to the World Health Organization, the restriction of sodium intake to less than 2.3 g/day, is one of the most cost-effective measures to improve public health (WHO, 2012). Therefore, with regards to limiting the daily intake of sodium, DFs such as RF, WS, and GR that contain lower levels could be considered to have better nutritional value than the other fish types.

Calcium is essential for bone and dental health, muscle contraction, nerve conduction, blood coagulation, enzyme activation, and hormone secretion (Quintaes & Diez-Garcia, 2015). The pattern of calcium contents of the DFs is comparable to that of sodium, with the FB, FA and FM having the highest levels. Bhowmik et al. (2022) reported a calcium content of 3.9 g/100 g in dried Punti (FB) powder while fresh anchovies contained 3.5 g/100 g (Ullah et al., 2022), both of which are lower than the values obtained for FB, FA, and FM in the present work. Using an estimated average consumption level of 15 g of DFs/day for adults (Bhowmik et al., 2022), the samples used in this study could meet 19.21–80.82% of the recommended dietary allowance (RDA, 1.3 mg/day) of calcium for adolescents (14–18 years of age) based on the United States Institute of Medicine Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, 1997. The daily intake of 15 g DFs can also contribute 39.5–84.3% of the calcium RDA (1000 mg/day) for pregnant women in India (Shlisky et al., 2022).

Table 1
Mineral composition of dried fishes[a].

Sample name	Phosphorus (g/100 g)	Potassium (g/100 g)	Calcium (g/100 g)	Sodium (g/100 g)	Magnesium (g/100 g)	Manganese (mg/1000 g)	Iron (mg/1000 g)	Copper (mg/1000 g)	Zinc (mg/1000 g)
Bombay duck	1.32 ± 0.13[c]	0.91 ± 0.30[d]	1.71 ± 0.05[d]	3.58 ± 2.95[abc]	0.25 ± 0.06[a]	12.93 ± 1.35[b]	306.16 ± 101.21[bc]	32.90 ± 37.32[a]	54.85 ± 11.32[c]
Ribbon fish	2.40 ± 0.8[ab]	1.22 ± 0.26[c]	3.79 ± 1.49[b]	3.01 ± 2.37[bc]	0.24 ± 0.04[a]	14.13 ± 2.64[b]	173.28 ± 79.44[cd]	5.71 ± 6.53[b]	56.26 ± 12.69[c]
White sardine	2.15 ± 0.12[b]	1.43 ± 0.19[b]	3.07 ± 0.25[bc]	2.44 ± 1.66[cd]	0.26 ± 0.01[a]	17.72 ± 1.28[b]	97.71 ± 22.61[d]	4.62 ± 2.05[b]	97.37 ± 8.77[b]
Freshwater barb	2.37 ± 0.24[ab]	0.79 ± 0.05[d]	5.02 ± 0.48[a]	5.32 ± 1.13[ab]	0.18 ± 0.02[a]	53.48 ± 44.53[a]	226.04 ± 23.48[bcd]	4.30 ± 1.15[b]	115.72 ± 6.22[b]
Ganges River sprat	2.23 ± 0.14[ab]	1.82 ± 0.17[a]	2.63 ± 0.55[cd]	0.41 ± 0.06[d]	0.17 ± 0.01[a]	29.18 ± 12.82[b]	407.84 ± 343.39[ab]	6.60 ± 0.77[b]	202.68 ± 38.4[a]
Fermented barb	2.56 ± 0.58[ab]	0.69 ± 0.05[d]	5.44 ± 0.88[a]	5.63 ± 1.71[a]	0.35 ± 0.40[a]	52.86 ± 14.87[a]	535.98 ± 159.19[a]	41.26 ± 29.72[a]	108.19 ± 10.22[b]
Fermented anchovies	2.77 ± 0.63[a]	0.71 ± 0.07[d]	5.62 ± 1.08[a]	4.51 ± 2.18[abc]	0.26 ± 0.02[a]	22.08 ± 7.03[b]	175.77 ± 26.05[cd]	3.05 ± 0.93[b]	64.02 ± 8.75[c]

[a] For each column, values with different letters are significantly different ($p < 0.05$).

The highest level of potassium was found in the GR ($p < 0.05$) while the fermented DFs (FM and FA) had the lowest values. However, the potassium levels in FM and FA are not significantly different from those of BD and FB. The 0.6 g/100 g potassium content in Punti powder (FB) as reported by Bhowmik et al. (2022), is consistent with the current results. However, Bhowmik et al. (2022) also reported 0.5 g/100 g potassium content for GR, which is lower than the values obtained in the current study. Dietary potassium benefits the reduction of blood pressure, especially among people with high-sodium diets (Weaver, 2013). In a longitudinal follow-up study (Mosallanezhad et al., 2023), a high sodium/potassium ratio (>0.8) was associated with an increased risk of cardiovascular diseases (CVD). In the current study, only the GR (sodium/potassium ratio of 0.22) met the suggested cut-off for a healthy sodium/potassium ratio. Therefore, regular consumption of GR may be used as excellent dietary practice to achieve a relatively healthy sodium/potassium ratio.

The highest phosphorus content was found in the FA while the lowest content was found in BD. The value obtained for the BD in this study is consistent with the 0.8 g/100 g reported by Nazir and Magar (1965). The values obtained for the DFs are similar to the 2.1–2.6 g/100 g reported by Bhowmik et al. (2022). Dietary phosphorus plays a crucial role in human bone growth, cell metabolism, signal transduction, phospholipid membrane integrity, and bone tissue construction, in addition to protein and nucleic acids (DNA and RNA) synthesis (Bird & Eskin, 2021). According to the United States Institute of Medicine Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, 1997, the estimated average requirement (EAR) for phosphorus in adolescents aged 9–18 years is 1.055 g/day. This requirement can be partially met by the consumption of 15 g of DFs used in this study by providing at least 17–51% of the phosphorus EAR. However, research has demonstrated that factors related to cardiovascular disease and metabolism were elevated in a mouse model that was fed a low dietary calcium/phosphorus ratio (0.76) for one week (Gutiérrez et al., 2020). In current study, the calcium/phosphorus ratio exceeded 0.76, with the highest value reaching 7.91 (in FA), indicating that the DFs may be effective in mitigating the risks associated with high phosphorus intake.

Magnesium is a relatively abundant element in the human body and is essential for the synthesis of nucleic acid substances and proteins, neuromuscular conduction, myocardial contractility, energy metabolism and immune system function (Al Alawi et al., 2021). Another study (Ciosek et al., 2021) has indicated that magnesium deficiency can adversely affect bone health by inhibiting the release of parathyroid hormone, which is essential for regulating calcium homeostasis. In the present study, the magnesium level of the DFs ranged from 0.17 to 0.35 g/100 g, showing no significant differences. This range is consistent with the values of 0.15 and 0.18 g/100 g reported for dried FB and GR, respectively (Bhowmik et al., 2022). According to the United States Institute of Medicine Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, 1997, the EAR for magnesium in the elderly population (individuals over 51 years of age) is 0.42 g/day for men and 0.32 g/day for women. Therefore, consumption of 15 g of DFs in this study can contribute 5–24% of the magnesium EAR for the elderly.

Zinc is one of the predominant micro-elements in DFs, and the highest content ($p < 0.05$) was found in GR, which is higher than the previously reported value of 128.6 mg/kg for whole dried Kachki (GR) powder (Bhowmik et al., 2022). The differences may be due to variations in environmental conditions, especially the available zinc intake from the water and feed sources. Zinc is crucial in mediating antioxidant and anti-inflammatory effects in the human body (Skalny et al., 2021). Zinc deficiency was proven to be related to neuropsychiatric and neurosensory disorders, skin lesions, acrodermatitis, hypogonadism and infertility, growth retardation, as well as thymic atrophy and immune dysfunction (Skalny et al., 2021). As reported by Bogard et al. (2015), about 45% of preschool-aged children and 57% of 15-49-year-old non-lactating/pregnant women were found to have inadequate zinc intake, highlighting a prevalent malnutrition issue in Bangladesh. The Food and Nutrition Board & Institute of Medicine (2001) recommended a dietary allowance (RDA) of 13 mg/day of zinc for lactating women aged 14–18 years. Regular consumption of 15 g of DFs in the present study can contribute at least 5–27% of the RDA for lactating mothers and therefore, may serve as good sources of dietary zinc. Furthermore, the high protein content in the DFs could enhance zinc solubility, thereby improving its bioavailability (Duan et al., 2023).

In the present study, the highest levels of manganese (52–53 mg/kg) were found in the *Puntius* spp, FB and FM. Notably, the results indicate that fermentation did not adversely affect the manganese level. Lower manganese contents of 3.4 and 4.1 mg/kg in whole Punti (FB) and Kachki (GR) powders, respectively were reported by Bhowmik et al. (2022). Adequate manganese intake is considered important for various physiological processes, including development and reproduction, bone and cartilage formation, wound healing, proper immune function as well as regulation of cellular energy, and blood sugar (Sachse et al., 2019). The recommended adequate intake (AI) of manganese for 14-50-year-old lactating women is 2.6 mg/day (Food and Nutrition Board & Institute of Medicine (2001)). Therefore, consumption of 15 g of the DFs in this study can fulfill at least 7–15% of this requirement. While manganese is an essential dietary element, the tolerable upper intake level (UL) for adults is 11 mg/day (Food and Nutrition Board & Institute of Medicine (2001)). Excessive manganese may disrupt the balance of other metals in the body, particularly iron, as both compete for intestinal absorption (Martins et al., 2020). Elevated levels of manganese absorption may be observed in individuals with iron deficiency anemia (Martins et al., 2020). Therefore, both FB and FM need to be consumed with caution to avoid excessive intake.

Iron is the most abundant microelement in the DFs, with the highest contents found in FM and GR. Bhowmik et al. (2022) reported an iron content of 328 mg/kg in whole FB powder, which is lower than some of the values obtained in the present study. Iron is a key prosthetic group in most iron-dependent enzymes and proteins, such as the heme. An adequate supply of heme is essential for functions as diverse as oxygen transport and storage, energy production, and drug metabolism (Fairweather-Tait & Sharp, 2021). However, iron-deficiency anemia affects approximately one-third of the world's population, especially the vulnerable groups, and is associated with adult lethargy, fatigue, and poor physical activity and work performance (Fairweather-Tait & Sharp, 2021). According to the Food and Nutrition Board & Institute of Medicine (2001), pregnant women aged 14–30 years have an iron RDA of 27 mg/day. At least 5% and up to 47% of the RDA of pregnant women can be met by consumption of 15 g of the DFs in present study, which make some of them excellent sources of dietary iron. Daily intakes of 15 g DFs can contribute 4.2–30% of the iron RDA (35 mg/day) for pregnant women in India (Kumar et al., 2022).

Among the DFs studied, the highest levels of copper were found in FM and BD. Given that FB exhibited a lower copper content, it is reasonable to suggest that fermentation may have led to copper enrichment in this fish type. Bhowmik et al. (2022) reported 5.3 and 3.7 mg/kg of copper in whole FB and Kachki (GR) powders, respectively, both of which are lower than the values obtained in the present study. Copper is essential as a component of various enzymes that facilitate cellular respiration, neurotransmitter transmission, and production of peptide hormones necessary for maintaining homeostasis (Zhen et al., 2022). The highest demand for copper occurs in pregnant and lactating women, with RDA values of 1 mg/day and 1.3 mg/day, respectively (Food and Nutrition Board & Institute of Medicine, 2001). Consumption of 15 g of DFs in this study can fulfill at least 3% and up to 93% of the RDA of copper, thereby serving as excellent sources of dietary copper.

3.3. Heavy metals

Fig. 2 shows the contents of five heavy metals (arsenic, cadmium, chromium, lead and mercury) in the DFs studied in this work. The general mechanism of heavy metal toxicity is through the generation of reactive oxygen species, the emergence of oxidative damage, and subsequent adverse health effects (Fu & Xi, 2020).

Comparing the relevant standard of 0.5 μg/g, mercury contents in the DFs did not exceed the allowable level. However, higher mercury levels were found in FM and FB, which are produced using the *Puntius* spp., and may be due to the fish samples being caught from the same environment. The results indicate that fermentation of this fish type did not reduce mercury content. In another study (Hoque et al., 2022), the mercury content in RF was found to be 0.08 μg/g, which is consistent with the results of this study. In contrast, much higher levels of mercury were reported in sun-dried BD (28.7 μg/g) and RF (48.3 μg/g), which could have been a reflection of environmental pollution (Rakib et al., 2021). Mercury is the deadliest heavy metal because of its extremely high affinity for antioxidant molecules (glutathione), which leads to a

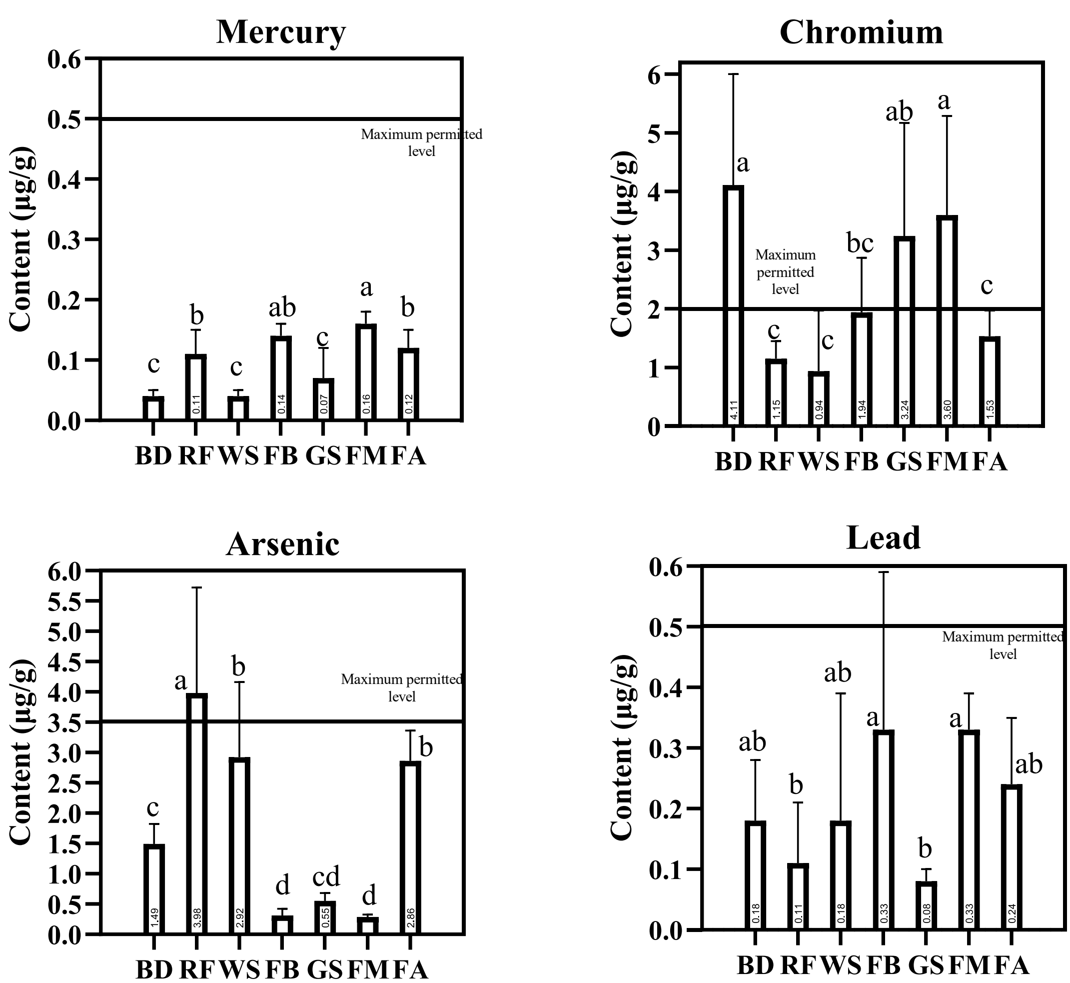

Fig. 2. Heavy metals profile of dried fishes. BD, Bombay duck; RF, Ribbon fish; WS, White sardine; FB, Freshwater barb; GS, Ganges River sprat; FM, Fermented barb; FA, Fermented anchovies. For each graph, bars with different letters have significantly ($p < 0.05$) different mean values. Lines represent permitted levels in foods: Mercury, Arsenic, and Lead maximum permitted levels, Health Canada, 2020; Chromium maximum permitted level, GB2762-2012, China, 2020.

decrease in antioxidant effectiveness and continues to be accumulated in the body due to lack of metabolic pathways for detoxification or complete removal (Houston, 2011). Long-term intake will lead to a decrease in the body's antioxidant capacity and an increased risk of negative health outcomes such as cardiovascular diseases and cerebrovascular accidents (Houston, 2011). However, BD, WS, and GR with significantly ($p < 0.05$) lower mercury levels could be a better choice for regular consumption when compared to FM, FB, FA, and RF.

Excessive levels of chromium in the human body produce some of the most serious damages among all heavy metals. Our work shows that the contents of chromium in BD, GR, and FM are significantly ($p < 0.05$) higher and exceeded the acceptable maximum permitted level. According to Rakib et al. (2021), the chromium contents of BD and RF were 7.06 and 9.34 μg/g, respectively, while Hossain et al. (2017) reported a value of 8.28 μg/kg for BD, all of which are higher than values obtained in the present study. In another study (Khatun et al., 2021), eight commonly eaten fish species (fresh) were collected from the Karnafuli River in Bangladesh, and found to contain an average chromium content of as high as 3.82 μg/g. It is expected that when converted to DFs, the chromium levels will exceed the maximum permitted content for human consumption. The toxicological mechanism is summarized as high-valent chromium ions that consume antioxidant molecules in the body and generate free radicals, which further increases the antioxidant burden of the body, resulting in damage to biologically active substances such as lipids, proteins, and DNA (DesMarias & Costa, 2019). Therefore, regular consumption of RF, FA, and WS, which have relatively lower chromium contents, are less likely to lead to chromium toxicity when compared to BD, GR, FB, and FM.

Among all study subjects, only the RF contained arsenic content that exceeded the maximum permitted level for human consumption. In a previous study (Kar et al., 2020), it was found that the arsenic content of RF was 22.27 μg/g, which is higher than the present findings. Therefore, the results suggest that the propensity to accumulate this heavy metal could depend on the amount present in the environment from where the fish was harvested. Inorganic arsenic, as a group-1 carcinogen, can inactivate up to 200 enzymes, especially those involved in cellular energy pathways as well as DNA replication and repair (Ratnaike, 2003). In addition, unbound arsenic ions can increase the body's oxidative burden and cause lipid and DNA damage (Ratnaike, 2003). Although Ratnaike (2003) suggested that organic arsenic is not toxic and tends to accumulate in fish, this does not endorse the safety of DFs with high arsenic contents. It has also been indicated that during the production and preservation processes of DFs, unscrupulous merchants spray pesticides on dried fish, which may contain carcinogenic inorganic arsenic (Kar et al., 2020; Khatun et al., 2021). However, it is unclear whether such a practice is responsible for the high arsenic level in RF.

The cadmium contents of all samples in this study are below the detection value, hence are less than the maximum permitted level for human consumption. This finding is consistent with the results reported in previous publications (Kar et al., 2020; Khatun et al., 2021). However, Hoque et al. (2022) reported up to 0.43 and 0.03 μg/g cadmium contents for BD and RF, respectively, which could be due to environmental contamination. Cadmium poisoning can induce diseases including osteoporosis, as well as damages to internal organs and the reproductive system among others (Genchi et al., 2020). Industrial pollution is believed to be the main source of cadmium contamination, and is relatively enriched in rice, vegetables, and shellfish that are consumed with DFs; therefore, the low cadmium content obtained in this study is desirable in minimizing toxicity (Genchi et al., 2020). The lead contents of all DFs are also lower than the maximum permitted level, which indicates a low risk of poisoning from regular consumption. Bhowmik et al. (2022) reported that the lead content of whole FB was 0.47 μg/g, which is higher than the levels found in this study. However, Hoque et al. (2022) reported lower levels of lead in BD (0.05 μg/g) and RF (0.05–0.136 μg/g). In addition, we found that FB and FM, the two DFs with higher fat contents (Fig. 1) and produced from the same species (*Puntius* spp.), had the highest lead contents, which may be due to the strong affinity of this heavy metal for hydrophobic environments (Wani et al., 2015). Lead poisoning manifests as delayed responses, irritability, and difficulty concentrating, as well as slowed motor nerve conduction and headaches (Wani et al., 2015). According to Wani et al. (2015), pregnant women and children are vulnerable groups that are more susceptible to lead poisoning, so these two groups should be cautious in consuming DFs like the FB and FM with high lead contents.

3.4. Amino acid composition and digestible essential amino acid score (DEAAS)

Proteins contain amino acids as the basic building blocks that are linearly linked by peptide bounds and are vital for normal body metabolic activities, which are needed for maintaining a healthy nutritional status. The predominant amino acids in DFs are glutamic acid + glutamine, followed by aspartic acid + asparagine, lysine and leucine (Table 2). Glutamic acid and aspartic acid are both key to the amino acid metabolism cycle in the body and are responsible for serving as neurotransmitters and participating in the energy cycle, playing an irreplaceable role in maintaining normal functioning of the immune system and intestines (David, 2012). Shah et al. (2020) indicated that glutamine is a critical part of amino acid metabolism because it is thought to be the fuel for regular activities of the immune system. Asparagine is recognized for its ability to prevent lymphocyte apoptosis and can promote an immune response (Li et al., 2007). Lysine, as one of the essential amino acids (EAA), is the most commonly deficient amino acid in developing countries where cereals are consumed as the staple food (Yang et al., 2022). Lysine is very important for human health, via maintaining the immune system, building the structural proteins of connective tissue, and controlling fatty acid metabolism (Yang et al., 2022). Therefore, supplementation of such cereal-based diets with DFs could ensure an adequate intake of lysine that meets the metabolic needs of the human body. Leucine is a promoter of cell growth and division, an anabolic mediator of protein metabolism, and has shown positive impacts on muscle protein synthesis, which can help to maintain healthy amounts of muscle proteins (Beaudry & Law, 2022). Therefore, the high leucine contents make the DFs suitable dietary choices for maintaining a healthy muscle mass. In addition, in this study, a relatively abundant arginine (up to 7.5 g/100 g protein) was found in the DFs, which indicates potential consumption could enhance cardiovascular health. This is because arginine is a substrate for nitric oxide production, especially when the human body is faced with pathogenic pressure. The amount of arginine that is synthesized by the body may not be able to meet the needs of adults and children, thus the DFs are desirable as exogenous sources that can adequately complement endogenous production (Wu et al., 2021).

Except for BD, all the DFs had EAA levels that are higher than 50 g/100 g protein of the total amino acids, which indicates potential to supply adequate amounts required for maintaining homeostasis. This is because EAA cannot be synthesized in the body and must come from dietary intakes. Findings in the present study are consistent with the EAA content reported in previous research on canned fish in oil from Poland (Usydus et al., 2009). In contrast, lower levels (<50 g/100 g protein) have been reported for several other types of DFs (Afé et al., 2021; Bhowmik et al., 2022). These differences could be due to variations in nutrient density in the environments from where the fish were caught. It is also worth noting that the contents of branched-chain amino acids (BCAA), namely leucine, isoleucine, and valine, account for about 17–18 g/100 g protein of the total amino acids, which is higher than the approximately 15 g/100 g previously reported value (Usydus et al., 2009). Like leucine, the other BCAA (isoleucine and valine) are also thought to contribute significantly to the normal functioning of the body's immune system. Lack of BCAA in the blood can impair the proliferation of lymphocytes, causing body vulnerability to bacteria and virus infections (Li et al., 2007). Bassit et al. (2002) reported that adding

Table 2
Amino acid composition (g/100 g protein) of dried fishes[a].

Amino acid[b]	Bombay duck	Ribbon fish	White sardine	Freshwater barb	Ganges River sprat	Fermented barb	Fermented anchovies
His	2.15 ± 0.27	2.52 ± 0.21	3.21 ± 0.45	2.86 ± 0.21	3.52 ± 0.43	3.09 ± 0.45	2.04 ± 0.40
Arg	6.29 ± 0.41	7.42 ± 0.29	7.00 ± 0.27	7.20 ± 0.43	7.51 ± 0.11	6.55 ± 0.97	5.57 ± 0.40
Thr	4.37 ± 0.16	5.03 ± 0.05	5.03 ± 0.09	4.93 ± 0.11	5.02 ± 0.06	3.97 ± 0.58	4.02 ± 0.52
Val	5.28 ± 0.12	5.76 ± 0.05	5.98 ± 0.10	5.78 ± 0.14	5.96 ± 0.04	5.95 ± 0.23	6.06 ± 0.17
Met	3.90 ± 0.70	3.78 ± 0.42	4.06 ± 0.31	3.20 ± 0.18	3.67 ± 0.22	3.46 ± 0.44	4.38 ± 1.09
Ile	4.69 ± 0.15	5.47 ± 0.11	5.41 ± 0.11	5.22 ± 0.13	5.28 ± 0.07	5.25 ± 0.20	5.41 ± 0.31
Leu	8.04 ± 0.37	8.98 ± 0.30	9.26 ± 0.11	8.95 ± 0.17	8.99 ± 0.10	8.74 ± 0.26	8.79 ± 0.38
Phe	4.29 ± 0.19	5.01 ± 0.12	5.32 ± 0.14	5.56 ± 0.21	5.28 ± 0.05	5.51 ± 0.45	5.48 ± 0.34
Trp	1.14 ± 0.10	1.13 ± 0.23	1.64 ± 0.12	0.90 ± 0.15	1.29 ± 0.04	0.74 ± 0.12	1.02 ± 0.19
Lys	8.29 ± 0.43	9.86 ± 0.26	9.82 ± 0.41	9.54 ± 0.33	9.81 ± 0.23	9.24 ± 0.95	9.11 ± 0.84
BCAA	18.02 ± 0.64^{ab}	18.09 ± 0.48^{ab}	18.57 ± 0.25^{a}	17.7 ± 0.37^{bc}	18.19 ± 0.11^{ab}	17.12 ± 0.48^{c}	17.61 ± 0.95^{bc}
EAA	48.44 ± 0.99^{d}	54.96 ± 0.65^{b}	56.76 ± 0.46^{a}	54.15 ± 0.73^{b}	56.33 ± 0.29^{a}	52.49 ± 1.19^{c}	51.89 ± 1.25^{c}
Ala	6.44 ± 0.18	6.77 ± 0.32	6.76 ± 0.09	7.13 ± 0.13	6.52 ± 0.12	9.26 ± 1.60	8.59 ± 1.05
Asx	9.37 ± 0.39	11.14 ± 0.47	10.81 ± 0.38	11.05 ± 0.53	11.08 ± 0.13	10.28 ± 0.90	10.68 ± 0.78
Cys	0.84 ± 0.07	1.04 ± 0.15	1.21 ± 0.08	0.97 ± 0.08	1.14 ± 0.05	0.94 ± 0.10	0.94 ± 0.13
Glx	15.84 ± 0.39	17.19 ± 0.29	16.78 ± 0.28	16.74 ± 0.54	16.52 ± 0.30	17.38 ± 1.56	18.87 ± 0.29
Gly	6.41 ± 0.27	6.89 ± 0.73	5.79 ± 0.24	8.15 ± 0.49	5.89 ± 0.22	11.08 ± 1.18	9.62 ± 2.49
Pro	4.67 ± 0.14	4.88 ± 0.32	4.48 ± 0.13	5.56 ± 0.27	4.70 ± 0.10	7.17 ± 0.64	6.57 ± 1.49
Ser	3.74 ± 0.10	4.52 ± 0.1	4.35 ± 0.09	4.72 ± 0.13	4.65 ± 0.08	4.01 ± 0.49	3.45 ± 0.27
Tyr	4.25 ± 0.35	4.25 ± 0.23	4.3 ± 0.13	4.22 ± 0.07	4.38 ± 0.16	3.82 ± 0.56	4.55 ± 0.36
DEAAS (%)	130.7 ± 4.7 (His)	132.0 ± 0.0 (Leu)	137.7 ± 1.5 (Val)	131.3 ± 1.5 (Val)	136.3 ± 0.6 (Leu, Val)	106.3 ± 11.5 (Trp)	113.0 ± 17.1 (His, Leu)

[a] For each row, values with different letters are significantly different ($p < 0.05$).
[b] BCAA = Branched-chain amino acids: leucine, isoleucine, valine; EAA = Essential amino acids: histidine, arginine, threonine, valine, methionine, isoleucine, leucine, phenylalanine, tryptophan, lysine; DEAAS = Digestible EAA score; Glx: glutamic acid + glutamine; Asx: aspartic acid + asparagine.

an appropriate amount of BCAA (6 g consisting of 60% leucine, 20% isoleucine and 20% valine) to athletes' diet can prevent tumors and stimulate lymphatic proliferation.

As described previously (FAO Expert Consultation, 2011), DEAAS is the lowest value of the EAA/reference amino acid digestion pattern, which reflects the quality of the limiting amino acids of the sample protein (i.e., to what extent DFs can meet the needs of a specific age group for the limited EAA). Therefore, DEAAS is used as an indicator of protein quality; the higher the value of DEAAS, the higher the quality of DFs protein, which can better meet the human body's demand for EAA (FAO Expert Consultation, 2011). As shown in Table 2, the DEAAS of all DFs are higher than 100%, which indicates they can meet 100% of the adult human's EAA requirement, and therefore can be considered as excellent sources of high-quality protein.

3.5. Free amino acids (FAAs)

Amino acids are not only the crucial components of proteins, but also significantly affect the taste of the food if functioning in the free or short peptide form (Zhao et al., 2016). Among all DFs, the RF had significantly ($p < 0.05$) lower total free amino acid (TFAA) content than the other DFs (Table 3). In contrast, FA, which is a fermented fish, had the highest TFAA levels, which may be related to the fermentation process where microbial and endogenous enzymes participate in protein hydrolysis to release FAAs (Liu et al., 2023). In a study on Egyptian salted-fermented fish (Rabie et al., 2009), the TFAAs of the product reached a 1.4-fold increment after the ripening stage, which is consistent with what we found in the current study. FB and FM are from the same species (*Puntius* spp.) but the TFAA content in the fermented FM is 1.2- to 2.8-fold higher than the unfermented FB. In current study, the most abundant FAA in DFs was glutamic + glutamine followed by alanine, leucine, and lysine. The presence of free Glu and Ala can increase consumers' acceptance of

Table 3
Free amino acid content (mg/100 g sample) of dried fishes[a].

	Bombay duck	Ribbon fish	White sardine	Freshwater barb	Ganges river sprat	Fermented barb	Fermented anchovies
His	6.9 ± 4.4^{d}	5.5 ± 1.2^{d}	109.2 ± 28.2^{b}	62.8 ± 11.0^{c}	331.4 ± 67.0^{a}	34.2 ± 10.9^{cd}	15.4 ± 8.4^{d}
Ser	44.6 ± 4.5^{c}	19.9 ± 14.1^{de}	72.5 ± 10.5^{b}	48.7 ± 16.9^{c}	97.0 ± 15.6^{a}	11.4 ± 7.5^{e}	32.0 ± 21.7^{cd}
Arg	52.2 ± 34.4^{cd}	19.9 ± 11.4^{e}	140.6 ± 14.6^{b}	59.9 ± 10.2^{c}	241.1 ± 31.9^{a}	29.9 ± 8.1^{de}	21.9 ± 7.7^{e}
Gly	78.8 ± 25.1^{b}	38.2 ± 31.4^{c}	88.9 ± 15.9^{b}	61.9 ± 13.6^{bc}	79.1 ± 8.6^{b}	81.2 ± 16.0^{b}	188.3 ± 35.7^{a}
Asx	132.0 ± 62.0^{b}	53.5 ± 28.4^{d}	111.3 ± 13.3^{bc}	77.1 ± 33.1^{cd}	83.7 ± 13.5^{bcd}	107.8 ± 48.0^{bc}	278.9 ± 47.4^{a}
Glx	312.3 ± 77.7^{b}	130.1 ± 90.6^{c}	300.7 ± 39.8^{b}	177.7 ± 91.3^{c}	224.8 ± 45.0^{bc}	324.9 ± 41.1^{b}	801.9 ± 176.0^{a}
Thr	61.1 ± 21.3^{b}	26.6 ± 18.1^{c}	110.9 ± 13.2^{a}	59.5 ± 26.2^{b}	95.1 ± 12.2^{a}	23.5 ± 13.8^{c}	62.4 ± 39.2^{b}
Ala	238.3 ± 56.8^{b}	99.1 ± 77.2^{c}	313.4 ± 34.5^{b}	132.3 ± 57.1^{c}	255.2 ± 29.8^{b}	289.3 ± 100.4^{b}	554.8 ± 165.0^{a}
Pro	72.4 ± 15.1^{c}	24.3 ± 16.8^{e}	104.4 ± 15.1^{b}	43.2 ± 15.2^{de}	108.0 ± 10.4^{b}	56.7 ± 8.4^{cd}	133.8 ± 34.7^{a}
Cys	7.3 ± 10.4^{abc}	1.1 ± 1.8^{c}	8.5 ± 4.3^{ab}	1.1 ± 1.1^{c}	7.3 ± 3.8^{abc}	2.5 ± 1.4^{bc}	12.2 ± 6.4^{a}
Lys	157.1 ± 100.4^{c}	41.7 ± 16.5^{d}	148.7 ± 12.6^{c}	125.3 ± 56.4^{c}	195.3 ± 27.9^{bc}	251.2 ± 27.7^{b}	404.8 ± 93.8^{a}
Tyr	260.2 ± 90.1^{a}	50.3 ± 38.4^{d}	148.0 ± 30.7^{bc}	55.6 ± 27.1^{d}	96.8 ± 21.7^{cd}	47.4 ± 19.9^{d}	184.1 ± 42.4^{b}
Met	50.3 ± 28.8^{b}	12.6 ± 8.2^{c}	50.2 ± 11.9^{b}	8.6 ± 5.8^{c}	46.2 ± 11.9^{b}	30.3 ± 10.6^{bc}	138.3 ± 50.3^{a}
Val	169.3 ± 34.0^{b}	54.1 ± 37.4^{d}	175.2 ± 23.0^{b}	87.8 ± 45.3^{cd}	132.1 ± 21.1^{bc}	139.4 ± 27.1^{bc}	339.9 ± 106.7^{a}
Ile	128.7 ± 36.3^{b}	37.5 ± 26.6^{c}	128.9 ± 16.5^{b}	68.4 ± 37.9^{bc}	93.5 ± 14.8^{bc}	116.9 ± 29.2^{b}	297.8 ± 130.1^{a}
Leu	234.2 ± 91.8^{bc}	58.0 ± 37.4^{d}	261.3 ± 31.4^{b}	125.7 ± 70.6^{cd}	216.1 ± 25.7^{bc}	219.2 ± 40.2^{bc}	574.2 ± 231.8^{a}
Phe	101.0 ± 48.0^{bc}	25.6 ± 16.1^{d}	105.1 ± 15.0^{b}	52.6 ± 30.0^{cd}	106.0 ± 11.9^{b}	92.4 ± 9.6^{bc}	234.6 ± 87.2^{a}
Trp	60.6 ± 26.8^{cd}	24.9 ± 15.9^{d}	99.8 ± 28.5^{ab}	28.3 ± 16.5^{d}	74.5 ± 20.4^{bc}	37.1 ± 26.5^{d}	120.7 ± 50.6^{a}
Total	2167.1 ± 589.7^{b}	722.9 ± 460.9^{d}	2477.6 ± 223.3^{b}	1276.5 ± 546.5^{cd}	2482.9 ± 235.0^{b}	1895.3 ± 225.2^{bc}	4396.1 ± 1215.6^{a}

[a] For each row, values with different letters are significantly different ($p < 0.05$).

DFs because of these amino acids have taste enhancement properties (Yin et al., 2022). Glu and Ala have umami (Bellisle, 1999) and sweetness (Yin et al., 2022) tastes, respectively that can work synergistically to enhance the taste of DFs. However, the presence of free Leu and Lys, could impart a negative taste in the DFs due to their bitter and unpleasant taste (Yin et al., 2022). The abundance of free Lys and presence of other FAAs will also contribute to the Maillard reaction during processing of the DFs, which promotes formation of browning and taste-enhancing compounds that can increase consumer acceptance (Shah et al., 2009). Apart from the above-mentioned effects, biogenic amines, a class of naturally occurring low molecular weight compounds usually with a strong odor, are also easily generated from FAA-rich environments (such as in the DFs) and constitute an important factor affecting the sensory quality of DFs (Rabie et al., 2009).

3.6. In vitro protein digestibility (IVPD)

In addition to the amino acid composition and amino acid score for determining protein quality, another crucial aspect that needs to be considered is digestibility. As the term suggests, digestibility reflects the extent to which a protein is broken down into smaller peptides; the higher the value, the better the protein can be absorbed by human body (Mohd Khairi et al., 2014). It was found that the IVPD of all the DFs are above 70%, with the highest digestibility ($p < 0.05$) being for RF (Table 4). The fermented DFs (FM and FA) had significantly ($p < 0\ 0.05$) lower IVPD than the other DFs, which suggests that fermentation may have produced substances or induced structural changes (e.g., increased formation of disulfide bonds) that interfered with proper enzymatic hydrolysis of the proteins. In general, the IVPD results obtained in this study are consistent with or higher than the 72% reported for freshly filleted RF though the differences in values may be due to the analysis methods and sample moisture content (Semedo Tavares et al., 2018). As discussed in a previous study (Bhat et al., 2022), procedures including salting and drying, which are inevitably used during DF production may lead to adverse structural changes in the protein due to oxygenation. For example, formation of protein-protein interactions, including cross-linking, aggregation, and disulfide bonds formation can reduce the sensitivity of the protein to digestive enzymes, thereby reducing the IVPD. On the other hand, DFs are usually subjected to further thermal cooking procedures before being consumed, which may be favorable to an increased protein digestibility. Semedo Tavares et al., 2018 compared the effects of different cooking methods (boiling, baking, and fry) on the digestibility of filleted RF, and found that cooking methods of all kinds significantly increased digestibility more than the values obtained for uncooked fish. Thus, it is believed that the DFs in present study may have the potential to achieve higher digestibility when cooked.

3.7. Protein composition

Three dominant compositions of fish muscle namely myofibrillar protein, sarcoplasmic protein and stroma protein make up about 60–65%, 30–35% and 3–5% of the total protein content, respectively (Ahmed et al., 2022). Table 4 lists the yields of sarcoplasmic and myofibrillar protein of each DF and their ratio. Overall, the yields of sarcoplasmic protein were higher than the myofibrillar protein and the high ratios indicate that the myofibrillar proteins were degraded during processing due to the activity of endogenous proteolytic enzymes such as cathepsins, serine proteases, collagenases and calpains (Yang et al., 2015). Degradation of the myofibrillar and sarcoplasmic proteins will not only affect their functional properties but also result in increased contents of short-chain peptides and free amino acids (Visessanguan et al., 2004), which may contribute to the taste and aroma of DFs.

3.8. Cholesterol content

Animal-based foods are the only sources of dietary cholesterol, so it is naturally present in human diets and tissues. Cholesterol is valued as an important component of cell membranes and a precursor to bile acids, steroid hormones, and vitamin D (Lecerf & de Lorgeril, 2011). In the present study, DFs had varied cholesterol contents, but with the GR having a significantly ($p < 0.05$) higher value than the other DFs (Table 4). Garcia-Vaquero et al. (2021) reported that Indian mackerel has a cholesterol content of 0.66 mg/g in fresh fish, which is about 10-fold lower than the results in current research. However, the higher content obtained in the current study may be due to the drying process, which concentrates cholesterol in the DFs. In the current study, there was no significant effect of fermentation on cholesterol contents, which contrasts the drop in cholesterol content of salted-fermented hoki roe as reported by Bekhit et al. (2018). The fishing season, eating habits and maturity of the fish affect the cholesterol content (Garcia-Vaquero et al., 2021), which could be responsible for the observed differences between the current work and the previous report on salt-fermented hoki roe.

The previous dietary guidance of >1 but <300 mg/day cholesterol for lowering cardiovascular disease risk has been shown to be inconsistent with results obtained from different populations. So far, scientists have suggested that dietary cholesterol can significantly increase total body cholesterol, however it cannot serve as a significant predictor of low-density lipoprotein cholesterol concentration, which has a strong correlation with cardiovascular disease risk (Carson et al., 2020). As indicated by Carson et al. (2020), the intake of cholesterol should be discussed within the general context of dietary patterns, which should be relatively low in cholesterol. Therefore, DFs, especially WS and GR with high cholesterol contents, should be consumed with fruits, vegetables, whole grains, low-fat or fat-free dairy products, nuts, seeds and liquid vegetable oils as part of an overall healthy diet. In addition, high salt content and processing of DFs may lead to lipid oxidation and the formation of harmful compounds called cholesterol oxidation products (COP), which may cause atherosclerosis, neurodegeneration, inflammation and carcinogenesis, and can be cytotoxic (Dantas et al., 2021). Since lipid oxidation is inevitable during dry fish production, especially sun-drying (Qiu et al., 2019), extended fermentation period may contribute to cholesterol content reduction and thereby reduce the COP

Table 4
Protein digestibility, and contents of vitamin B_{12}, cholesterol, and muscle proteins of dried fishes[a].

Fish sample	Vit. B_{12} Content (μg/g)	Cholesterol (mg/g)	Sarcoplasmic protein (g/100 g)	Myofibrillar protein (g/100 g)	Sarcoplasmic/Myofibrillar Ratio (g/100 g)	In vitro protein digestibility (%)
Bombay duck	0.05 ± 0.03^{bc}	5.64 ± 0.41^{cd}	11.89 ± 5.51^{cd}	2.32 ± 0.34^{e}	$4.96{:}1^{a} \pm 1.78$	78.9 ± 2.17^{bc}
Ribbon fish	0.09 ± 0.05^{b}	5.73 ± 0.78^{cd}	6.89 ± 2.84^{e}	3.49 ± 0.72^{de}	$1.91{:}1^{cde} \pm 0.51$	84.0 ± 1.07^{a}
White sardine	0.07 ± 0.01^{bc}	8.24 ± 0.63^{b}	16.68 ± 3.16^{ab}	10.43 ± 0.95^{a}	$1.6{:}1^{de} \pm 0.30$	79.87 ± 0.51^{b}
Freshwater barb	0.06 ± 0.01^{bc}	5.74 ± 0.33^{cd}	12.98 ± 3.33^{bcd}	6.11 ± 1.54^{c}	$2.12{:}1^{cd} \pm 0.04$	78.24 ± 1.75^{bc}
Ganges River sprat	0.21 ± 0.03^{a}	12.40 ± 1.49^{a}	9.25 ± 1.71^{de}	8.16 ± 0.51^{b}	$1.14{:}1^{e} \pm 0.28$	77.64 ± 0.39^{c}
Fermented barb	0.04 ± 0.02^{c}	4.88 ± 0.57^{d}	18.5 ± 1.55^{a}	7.21 ± 1.39^{bc}	$2.63{:}1^{c} \pm 0.51$	73.74 ± 2.38^{d}
Fermented anchovies	0.07 ± 0.02^{bc}	5.88 ± 0.48^{c}	14.15 ± 1.07^{bc}	4.23 ± 1.32^{d}	$3.55{:}1^{b} \pm 0.90$	72.63 ± 1.65^{d}

[a] For each column, values with different letters are significantly different ($p < 0.05$).

content.

3.9. Vitamin B_{12} content

Vitamin B_{12} is an important water-soluble compound of animal and microbial origins (Obeid et al., 2019). Vitamin B_{12} deficiency is widespread around the world and is particularly common among people who, for various reasons such as income, ethics and lifestyle, have low consumption of animal-based foods (Obeid et al., 2019). Among all the DFs, the GR had a significantly higher vitamin B_{12} content (Table 4). The findings in this study are higher than the results reported in previous studies of fresh BD and GR with 0.015 μg/g (Nordhagen et al., 2020) and 0.036 μg/g (Bogard et al. 2015) contents, respectively. The higher levels obtained in the current study could be due to the drying process, which concentrates the vitamin B_{12} content based on unit-weight of the product. According to the European Food Safety Authority (Obeid et al., 2019), the recommended adequate intake of vitamin B_{12} is 4.0 μg/day for adults. Higher adequate intakes (4.5–5.0 μg/day of vitamin B_{12}) for pregnant women and lactating women were suggested (Obeid et al., 2019), which could be achieved by consuming approx. 25 g or more of GR.

Table 5
Fatty acid composition (g/100 g of total fatty acids) of dried fishes[a].

Fatty acid	Bombay duck	Ribbon fish	White sardine	Freshwater barb	Ganges River sprat	Fermented barb	Fermented anchovies
Caprylic acid (C8:0)	n.d. [b]	n.d. [b]	n.d. [b]	0.005 ± 0.002	n.d. [b]	0.005 ± 0.002	n.d. [b]
Capric acid (C10:0)	0.009 ± 0.001	0.003 ± 0.001	0.008 ± 0.001	0.015 ± 0.006	0.011 ± 0.006	0.011 ± 0.002	0.008 ± 0.002
Lauric acid (C12:0)	0.342 ± 0.116	0.061 ± 0.01	0.189 ± 0.057	0.248 ± 0.029	0.159 ± 0.017	0.402 ± 0.114	0.321 ± 0.049
Myristic acid (C14:0)	5.97 ± 1.045	3.804 ± 0.778	5.22 ± 0.544	2.219 ± 0.331	3.554 ± 0.276	2.094 ± 0.196	4.881 ± 0.51
Pentadecylic acid (C15:0)	0.588 ± 0.073	0.907 ± 0.071	1.023 ± 0.052	1.13 ± 0.295	1.212 ± 0.105	1.13 ± 0.136	0.81 ± 0.13
Palmitic acid (C16:0)	35.057 ± 2.837	31.788 ± 4.855	29.193 ± 0.815	26.95 ± 1.277	28.28 ± 1.304	26.513 ± 0.619	31.792 ± 0.702
Margaric acid (C17:0)	0.765 ± 0.166	1.342 ± 0.163	1.332 ± 0.082	1.928 ± 0.166	2.224 ± 0.11	1.727 ± 0.206	1.12 ± 0.137
Stearic acid (C18:0)	7.094 ± 1.402	13.757 ± 2.014	9.974 ± 0.746	9.889 ± 0.557	9.359 ± 0.679	9.997 ± 0.455	10.941 ± 0.584
Arachidic acid (C20:0)	0.405 ± 0.076	0.541 ± 0.111	0.455 ± 0.031	0.389 ± 0.084	0.308 ± 0.018	0.378 ± 0.029	0.703 ± 0.061
Behenic acid (C22:0)	0.595 ± 0.147	0.448 ± 0.116	0.523 ± 0.126	0.215 ± 0.027	0.316 ± 0.008	0.174 ± 0.018	0.708 ± 0.054
Lignoceric acid (C24:0)	0.544 ± 0.172	0.897 ± 0.203	1.084 ± 0.178	0.153 ± 0.035	0.724 ± 0.178	0.103 ± 0.014	0.596 ± 0.156
∑SFA[b]	51.367 ± 3.385[ab]	53.547 ± 6.018[a]	48.998 ± 0.229[bc]	43.135 ± 1.413[de]	46.145 ± 1.424[cd]	42.528 ± 1.32[e]	51.88 ± 1.729[ab]
Myristoleic acid (C14:1)	0.079 ± 0.024	0.019 ± 0.014	0.011 ± 0.002	0.059 ± 0.024	0.114 ± 0.051	0.05 ± 0.017	0.019 ± 0.005
Palmitoleic acid (C16:1)	0.274 ± 0.03	0.24 ± 0.064	0.167 ± 0.016	n.d. [b]	n.d. [b]	n.d. [b]	0.23 ± 0.046
Palmitolaidic acid (C16:1t)	10.419 ± 1.644	4.544 ± 1.917	5.692 ± 0.629	3.947 ± 1.014	3.906 ± 0.558	4.474 ± 0.699	6.682 ± 0.931
Oleic acid (C18:1)	12.889 ± 1.492	13.301 ± 2.624	5.914 ± 0.369	30.37 ± 4.488	7.702 ± 0.351	31.699 ± 2.432	15.135 ± 1.386
Vaccenic acid (C18:1n7c)	2.894 ± 0.201	2.974 ± 0.382	3.578 ± 0.22	2.675 ± 0.516	2.891 ± 0.284	2.423 ± 0.214	3.419 ± 0.083
Eicosenoic acid (C20:1)	0.614 ± 0.138	0.438 ± 0.069	0.226 ± 0.036	0.853 ± 0.09	0.424 ± 0.06	0.792 ± 0.063	0.534 ± 0.102
Erucic acid (C22:1)	0.065 ± 0.031	0.06 ± 0.011	0.05 ± 0.017	0.142 ± 0.134	0.093 ± 0.006	0.024 ± 0.006	0.098 ± 0.054
Nervonic acid (C24:1)	0.481 ± 0.175	1.248 ± 0.325	0.798 ± 0.119	0.126 ± 0.051	0.401 ± 0.075	0.071 ± 0.02	0.592 ± 0.141
∑MUFA[b]	27.713 ± 2.033[b]	22.824 ± 4.81[c]	16.436 ± 1.123[d]	38.172 ± 3.73[a]	15.532 ± 0.731[d]	39.533 ± 1.86[a]	26.709 ± 1.88[b]
Linoleic acid (n-6, C18:2)	0.765 ± 0.06	1.109 ± 0.138	1.028 ± 0.08	7.154 ± 0.326	4.482 ± 1.343	8.295 ± 1.282	1.818 ± 0.704
γ -Linolenic acid (n-6, C18:3n6)	0.268 ± 0.062	0.213 ± 0.105	0.282 ± 0.005	0.324 ± 0.072	0.828 ± 0.214	0.553 ± 0.199	0.402 ± 0.078
α-Linolenic acid (n-3, C18:3n3)	0.425 ± 0.127	0.257 ± 0.071	0.568 ± 0.185	3.548 ± 0.88	4.644 ± 1.213	2.945 ± 0.455	0.768 ± 0.332
Eicosadienoic acid (n-6, C20:2)	0.187 ± 0.035	0.188 ± 0.034	0.185 ± 0.033	0.449 ± 0.035	0.42 ± 0.19	0.402 ± 0.038	0.212 ± 0.021
Eicosatrienoic acid (n-6, C20:3n6)	0.133 ± 0.016	0.215 ± 0.034	0.171 ± 0.006	0.445 ± 0.11	0.731 ± 0.333	0.41 ± 0.066	0.215 ± 0.037
Arachidonic acid (n-6, C20:4)	2.49 ± 0.601	2.728 ± 1.083	3.693 ± 0.978	2.165 ± 0.341	3.932 ± 0.482	1.892 ± 0.177	2.081 ± 0.432
Ecosatrienoic acid (n-3, C20:3n3)	0.144 ± 0.055	0.081 ± 0.006	0.084 ± 0.006	0.263 ± 0.102	0.428 ± 0.126	0.16 ± 0.035	0.124 ± 0.015
Eicosapentaenoic acid, EPA (n-3, C20:5)	4.887 ± 0.623	2.327 ± 0.405	6.541 ± 0.675	0.853 ± 0.189	5.143 ± 1.806	0.651 ± 0.099	4.119 ± 0.901
Docosadienoic acid (n-6, C22:2)	0.017 ± 0.011	0.019 ± 0.005	0.014 ± 0.005	0.016 ± 0.005	0.015 ± 0.006	0.01 ± 0.002	0.015 ± 0.003
Adrenic acid (n-6, C22:4)	0.275 ± 0.089	0.58 ± 0.207	0.276 ± 0.06	0.308 ± 0.045	0.207 ± 0.034	0.31 ± 0.036	0.369 ± 0.131
Docosapentaenoic acid (n-6, C22:5n6)	0.705 ± 0.166	1.354 ± 0.504	1.302 ± 0.14	0.494 ± 0.068	2.55 ± 0.353	0.48 ± 0.086	0.788 ± 0.205
Docosapentaenoic acid, DPA (n-3, C22:5n3)	0.977 ± 0.168	1.476 ± 0.12	0.951 ± 0.02	0.608 ± 0.185	1.274 ± 0.231	0.457 ± 0.08	1.655 ± 0.446
Docosahexanoic acid, DHA (n-3, C22:6n3)	9.647 ± 2.249	13.083 ± 3.258	19.474 ± 0.311	2.064 ± 0.749	13.671 ± 1.543	1.373 ± 0.341	8.847 ± 1.404
∑PUFA[b]	20.919 ± 3.755[cd]	23.63 ± 5.27[c]	34.568 ± 1.257[b]	18.688 ± 2.34[d]	38.324 ± 1.034[a]	17.936 ± 2.639[d]	21.41 ± 2.462[cd]
UFA[b]	48.632 ± 3.386[de]	46.454 ± 6.017[e]	51.004 ± 0.229[cd]	56.861 ± 1.416[ab]	53.855 ± 1.424[bc]	57.469 ± 1.323[a]	48.119 ± 1.729[de]
∑n-3	16.08 ± 3.016[b]	17.223 ± 3.481[b]	27.617 ± 0.714[a]	7.334 ± 2.05[c]	25.159 ± 2.519[a]	5.585 ± 0.975[c]	15.512 ± 2.159[b]
∑n-6	2.349 ± 0.298[d]	3.678 ± 0.73[c]	3.258 ± 0.148[cd]	9.189 ± 0.148[b]	9.233 ± 1.717[b]	10.459 ± 1.504[a]	3.817 ± 0.659[c]
n-3/n-6	6.806 ± 0.483[b]	4.68 ± 0.053[c]	8.493 ± 0.5[a]	0.799 ± 0.226[e]	2.863 ± 0.895[d]	0.532 ± 0.022[e]	4.211 ± 1.13[c]
PUFA/SFA	0.412 ± 0.102[c]	0.452 ± 0.143[c]	0.706 ± 0.028[b]	0.432 ± 0.041[c]	0.832 ± 0.047[a]	0.423 ± 0.072[c]	0.414 ± 0.056[c]

[a] For each row, values with different letters are significantly different ($p < 0.05$).
[b] SFA: saturated fatty acid; PUFA: polyunsaturated fatty acid; MUFA: monounsaturated fatty acid; n.d.: not detected.

3.10. Fatty acid composition

The quality of fish lipids can be evaluated based on the levels of fatty acids such as saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA), n-3, n-6, n-3/n-6 ratio and PUFA/SFA, which are shown in Table 5. Among all eleven detected SFA, the content of palmitic (C16:0) was highest, accounting for 26–35 g/100 g of total fatty acids. In terms of the predominant MUFA, palmitoleic (C16:1) and oleic (C18:1) were enriched in all DFs, though oleic contents of FB and FM were the highest. The similar oleic acid values for FB and FM is because they are the same species of fish, but the results indicate that the fermentation process did not have a negative effect on oleic acid content of this fish type. For the n-6 fatty acids, arachidonic and linoleic acids were the most abundant. As for n-3 fatty acids, docosahexaenoic (DHA) and eicosapentaenoic acid (EPA) were the most abundant, especially in GR and FA. The GR, FB, and FM also had the highest levels of α-linolenic acid, another n-3 fatty acid. The fatty acid profiles of DFs in the current study are consistent with the 23.84 g/100 g palmitic, 25.11 g/100 g oleic, 3.88 g/100 g palmitoleic, and 7.85 g/100 g linoleic, 2.71 g/100 g arachidonic, 3.36 g/100 g α-linolenic reported for FB in a previous study (Bhowmik et al., 2022). The same study also reported the fatty acid profile of whole GR as containing 30.12 g/100 g palmitic, 8.92 g/100 g oleic, 5.26 g/100 g palmitoleic, 3.63 g/100 g arachidonic, 8.64 g/100 g DHA, and 3.58 g/100 g EPA, which are similar to the findings of the current study.

The nutritional value of fish oil is widely recognized because it is rich in a variety of n-3 fatty acids that are beneficial to health. DHA and EPA, as the main n-3 fatty acids of DFs in this study, are recommended (dietary intake 0.25–0.5 g/day) by the European Food Safety Authority (EFSA, 2010) for their ability to prevent cardiovascular diseases. In addition, a recent study (Islam et al., 2021) indicated that EPA and DHA have positive effects such as enhanced formation of the nervous system (especially in the human brain and retina) and reduction of liver steatosis. In this sense, the abundance of DHA and EPA in WS, BD, RF, GR, and FA makes them excellent sources of n-3 fatty acids. On the other hand, α-linolenic acid, as the main n-3 fatty acid in FB and FM, can be added to the diet as a countermeasure against cardiovascular diseases for people lacking DHA and EPA (Campos et al., 2008).

According to Coskuntuna et al. (2015), a dietary n-3/n-6 ratio below 0.25 will promote cardiovascular diseases. Nindrea et al. (2019) suggested that increasing the n-3/n-6 ratio can prevent breast cancer, especially in Western populations (whose dietary n-3 intake is relatively low compared to that of Asians). The n-3/n-6 ratio of all DFs in this study is higher than 0.5, among which WS has a significantly ($p < 0.05$) higher ratio than the other DFs. Therefore, adding DFs with high n-3/n-6 ratios such as WS, to the diet could reduce the excessive n-6 fatty acid intake associated with most human diets to a healthy level. The PUFA/SFA ratio is also an important indicator to evaluate the quality of fish oil, with the value for health benefits recommended to be higher than 0.4 (Wood et al., 2008). Phillips et al. (2012) indicated that a low dietary PUFA/SFA ratio (<0.38) will further aggravate body mass index (≥25 kg/m^2), which constitutes a risk for abdominal obesity. In this study, though the PUFA/SFA ratios of the DFs are higher than 0.4, those of WS and GR are the highest ($p < 0.05$). Therefore, in the sense of reducing obesity risk, regular consumption of WS and GR as part of the human diet could be a useful approach. However, higher PUFA/SFA levels of 1.10 and 1.38 have been reported for *Clarias gariepinus* dried fish produced with drum kiln and eco-friendly kiln, respectively (Ogunbambo, 2020).

4. Limitations

The processing details of the DFs in this experiment are limited, as the duration of drying and the quantity of salt used were not specified by the fish vendors. Because the nutrient composition of DFs is significantly affected by the capture season, life cycle, food availability, living environment, and processing conditions of the fishes, a comprehensive sampling is required. However, the sampling locations of this study are limited to Cox's Bazar, Dhaka, Mymensingh, Sylhet and the information regarding the capture season are missing. Furthermore, the estimate of the contribution of consuming 15 g of DFs to the nutritional requirements of the target population may be dependent on the cooking methods employed. Despite these limitations, the data presented in this study hold significant value, as they provide species-specific estimates of various nutrients that can serve as a basis for comparison in future research, particularly given the lack of existing comprehensive nutritional composition data for DFs that are consumed in Bangladesh.

5. Conclusions

In this study, two indigenous small fishes, WS and GR have shown particular efficiency in meeting the daily dietary requirements for calcium, potassium, manganese, iron, copper, zinc, essential amino acids (EAA), EPA, DHA, and vitamin B_{12} in vulnerable populations, with minimal overdose concerns. In contrast, the benefits of FB consumption (indigenous small fish) may be offset by its high sodium content, low protein content and quality, low vitamin B_{12} content and less preferable fatty acids profile when compared with WS and GR. However, fermentation led to improved protein digestibility and production of free amino acids that could contribute to better eating quality of the DFs. The medium/large DFs (BD and RF) provide moderate nutritional benefits but have high cholesterol and saturated fatty acids, which may reduce their overall advantages. Overall, increased consumption of WS and GR could be recommended for enhanced nutritional status of consumers of DFs.

CRediT authorship contribution statement

Huan Sun: Writing – original draft, Methodology, Formal analysis, Data curation. **Derek S. Johnson:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Rotimi E. Aluko:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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