NUTRITIONAL COMPOSITION OF DRIED FISHES FROM BANGLADESH AND FUNCTIONAL PROPERTIES OF THEIR PROTEIN ISOLATES

By

Huan Sun

A thesis Submitted to the Faculty of Graduate Studies of
the University of Manitoba
in partial fulfilment of the requirements of the degree of

Master of Science

Department of Food and Human Nutritional Sciences
University of Manitoba
Winnipeg, Manitoba Canada

ABSTRACT

Dried fishes (DFs) are considered to be rich in protein, fat, ash and easy to store as well as transport at room temperature. However, they have been neglected for a long time with respect to their nutritional quality, consumption safety and their potential as an innovative food ingredient. To alleviate the serious public health challenge posed by malnutrition and find new possibilities for the food industry, it is particularly important to study the nutritional profile of dried fish and explore their functional characteristics to pave the way for subsequent industrial applications. Therefore, this study explored the nutrient composition of seven different DFs (Bombay duck: BD, *Harpadon nehereus*; ribbon fish: RF, *Trichiurus iepturus*; white sardine: WS, *Escualosa thoracata*; freshwater barb: FB, *Puntius spp.*; Ganges River sprat: GR, *Corica soborna*; fermented barb: FM, *Puntius spp.*; fermented anchovies: FA, *Setipnna spp.*) collected from four cities (Cox's Bazar: C; Dhaka: D; Mymensingh: M; Sylhet: S) in Bangladesh. In addition, further investigation was conducted on determining the functional properties of dried fish protein isolates (DFPIs) from Dhaka at neutral pH (7.0), thereby to broaden the value-addition of DFs and explore potential applications of the isolated proteins as ingredients in the food industry. We found that two small indigenous DFs, WS and GR, showed good potential as a protein source because both of them contained more than 75% protein, in which the essential amino acids (EAA) content accounted for 50% of the total amino acids, and the scores of various individual EAAs exceeded 100. In addition, there were significantly ($p<0.05$) lower contents of sodium and higher potassium in WS and GR, making them good sources of these minerals. An excellent fatty acid profile was detected in WS based on the high levels of EPA (6-7%), DHA (~19%) and other n-3 fatty acids (total n-3 fatty acid: 32-35%), making it a great source of health-promoting essential fatty acids. All the DFs analyzed in this study are good

sources of vitamin B_{12}. However, the presence of high levels of heavy metals in several of the DFs, except for WS, is not desirable due to potential adverse effects on human health. In addition, a relatively high cholesterol content (0.17-0.25 μg/g) in GR also limits its promotion potential among consumers as a source of healthy nutrients. In terms of DFPIs' functional proprieties, it was found that the high concentration of NaCl contained in DFs increased the ionic strength of the extraction environment and caused the isoelectric point (IP) to move towards to acidic direction (pH 4.5). Under neutral pH conditions, BD-D exhibited higher solubility (85.03±1.06%), relatively stable heat coagulability (6.23 ± 0.41 %), excellent water/oil holding capacity (water holding: 7.00 ± 0.47 g/g; oil holding: 20.13 ± 0.14 ml/g), as well as strong gelling ability (least gel concentration: 3%) and emulsifying properties (oil droplets size of 2-3.5 μm; emulsion stability: 70-100 %). Far-UV CD results reflect that β-sheet (20-35%) and random coil (35-46%) are the predominant secondary structures of DFPIs, indicating highly denatured structures. The almost zero ellipticity in the near-UV spectrum also implies that except the GR, most of the tertiary structures in DFPIs are also unfolded. The polypeptide profile lacked the characteristic band of myofibrillar protein at 200 kDa. Instead, the broad bands at 37-25 and 15-10 kDa indicate that the myofibrillar and sarcoplasmic protein of DFPIs have been degraded into shorter peptides with low molecular weights.

ACKNOWLEDGEMENT

I am very grateful for the opportunity to have a supervisor like Dr. Rotimi Aluko who beyond excellent supervision, has been a mentor and a constant source of support. I would like to thank my advisory committee - Dr. Derek Johnson and Mr. Alphonsus Utioh for their very useful guidance and contributions in completing my thesis.

I also acknowledge Dried Fish Matters for their financial support towards my research.

My sincere gratitude to the late Dr. Mostafa Hossain (Bangladesh Agricultural University) for his assistance in suggesting the product mix and getting the samples for this study.

My sincere gratitude goes to Dr. Nancy Asen as well as other members of Dr. Aluko's research team for numerous ways they have helped me during my program.

Finally, words cannot express my gratitude to my parents, Mr. Jianying Sun and Ms. Huirong Zhang, as well as the rest of my family, friends, and relatives for the huge role they have played in getting me to this point.

FOREWARD

This thesis was compiled using the manuscript format and it consists of two manuscripts, which follow immediately after the general introduction and literature review chapters. Manuscript 1 determined the nutritional quality of dried fish from Bangladesh. Manuscript 2 examined the structural and functional properties of dried fish protein isolates. A transition statement is provided after manuscript 1 to link it to the next chapter for a consistent flow. The last chapter provides the overall summary of the study and possible future directions.

TABLE OF CONTENTS

LIST OF TABLES

Chapter Two

Chapter Three

Chapter Four

concentration and surface hydrophobicity of dried fish protein isolates

Table 3. Secondary structure fractions of dried fish protein isolate at pH 7.0

LIST OF FIGURES

Chapter Four

LIST OF ABBREVIATIONS

(-S-S-) - Disulfide bonds

AA - Amino acids

AI - Adequate intake

Ala - Alanine

ANOVA - Analysis of variance

ANS - 1-anilino-8-naphthalenesulfonate

As - Arsenic

Asn - Asparagine

Asp - Aspartic acid

BCAA - Branched-chain amino acids

BD - Bombay duck

BF_3 - Boron trifluoride

C - Cox's Bazar

CA - Canada

Ca - Calcium

$CaCl_2$ - Calcium chloride

Cd - Cadmium

CD - Circular dichroism

CDFs - Cox's Bazar dried fishes

Cl^- - Chloride iron

COP - Cholesterol oxidation products

Cr - Chromium

Cu - Copper

CVD - Cardiovascular diseases

Cys - Cystine

D - Dhaka

DDFMs - Defatted Dried fishmeal

DDT - Dichlorodiphenyltrichloroethane

DDW - Double distilled water

DEAAS - The digestible essential amino acid score

DF - Dried fish

DFM - Dried fishmeal

DFPIs - Dried fish protein isolates

DHA - Docosahexanoic acid

DM - Dry matter

DNA - Deoxyribonucleic acid

DPA - Docosapentaenoic acid

EAA - Essential amino acids

EAR - Estimated average requirement

EC - Emulsifying capacity

EFSA - European Food Safety Authority

EPA - Eicosapentaenoic acid

ES - Emulsion stability

FA - Fermented anchovy

FAAs - Free amino acids

FAO - Food and Agricultural Organization

FB - Freshwater barb

Fe - Iron

FI - Fluorescence intensity

FM - Fermented barb

FPI - Fish protein isolate

GC - Gas chromatography

Gln - Glutamine

Glu - Glutamic acid

Gly - Glycine

GR - Ganges River sprat

HAD - Hot air drying

HC - Heat coagulability

Hg - Mercury

His - Histidine

H_o - Surface hydrophobicity

HPD - Heat pump drying

HPLC - High-performance liquid chromatography

Ile - Isoleucine

IP - Isoelectric point

IVPD - In vitro protein digestibility

K - Potassium

KOH - Potassium hydroxide

LDL - Low-density lipoprotein

LDPE - Low-density polyethylene

Leu - Leucine

LGC - Least gelation concentration

Lys - Lysine

M - Mymensingh

Met - Methionine

Mg - Magnesium

MHC - Myosin heavy chain

ML - Maximum level

MPs - Microplastics

MRL - Maximum residue limit

MT - Metric tons

MUFA - Monounsaturated fatty acid

MW - Molecular weight

n-3 LC-PUFA - n-3 long-chain polyunsaturated fatty acids

Na - Sodium

Na_2SO_4 - Sodium sulfate anhydrous

NAA - Non-essential amino acids

NaCl - Sodium chloride

NaOH - Sodium hydroxide

nd - Not detected

OHC - Oil holding capacity

P - Phosphorus

PA - Polyamide

Pb - Lead

Phe - Phenylalanine

Pro - Proline

PS - Polystyrene

PUFA - Polyunsaturated fatty acid

PVC - Polyvinyl chloride

RDA - Recommended dietary allowance

RF - Ribbon fish

S - Sylhet

SDFs - Sylhet dried fishes

SDS - Sodium dodecyl sulfate

SDS-PAGE - Sodium dodecyl sulphate-polyacrylamide gel electrophoresis

Ser - Serine

SFA - Saturated fatty acid

SH - Sulfhydryl group

SLs - Sampling locations

TFAA - Total free amino acid

Thr - Threonine

Trp - Tryptophan

Tyr - Tyrosin

UL - Tolerable upper intake level

UV - Ultraviolet–visible

Val - Valine

VFD - Vacuum freeze drying

VSD - Vacuum spray drying

W_1 - Glass tube weight

W_2 -Weight of the tube with lipids

WHC - Water holding capacity

WHO - World Health Organization

WS - White sardine

Zn - Zinc

CHAPTER ONE

1 INTRODUCTION

Fish is one of the representatives of nutritious foods as it is rich in a variety of indispensable nutrients, which include n-3 fatty acids, essential amino acids, minerals, vitamins, etc. (Rahman et al., 2012). Among the nutrients, fish oil or specifically the n-3 fatty acids have shown superior ability in disease prevention and health maintenance. Scientists have found that in addition to cardiovascular and cerebrovascular diseases, fish oil also has benefits for the central nervous system, proinflammatory cytokines and skeletal muscle health (Lewis et al., 2020). In addition, studies on health benefits of fish protein, peptides, or hydrolysates, even in their early stages of development, have revealed a significant role in almost as many fields as fish oils (Ahmed et al., 2022; Khalili Tilami & Sampels, 2018). There are also studies that use fish bone powder as a calcium fortifier in cookies or in other food systems (Junianto et al., 2022; Njoroge & Lokuruka, 2020). However, in the modern consumer and industrial environment, many by-products of fisheries are not fully utilized or are even wasted (Gehring et al., 2011).

Facing the challenge of continued growth of the world's population, there is year-by-year increasing pressure to nourish the population, and accordingly decrease the malnutrition rate in many developing countries (Gehring et al., 2011). In 2017, the number of undernourished people was approximately 821 million, of which approximately 124 million were in a state of acute malnutrition emergency in more than 51 countries and regions (Siddhnath et al., 2022). As nutritional shortages are closely related to poverty, nutrition-related health problems are more prominent in poorer countries. In India, about 20% of children under the age of 5 suffer from wasting due to malnutrition, a condition in which their weight is low relative to height

(Siddhnath et al., 2022). Various degrees of wasting, underweight, and stunting in children have also been reported in Bangladesh (Hossain et al., 2020). In addition to children, other vulnerable groups such as pregnant/lactating women and the elderly also face the challenge from malnutrition, including lack of zinc, iron, calcium, vitamin A, and vitamin B_{12} (Fiedler et al., 2016). To alleviate hunger and malnutrition, it is rational to bring those previously neglected or underestimated fishery by-products back on the radar as a potential solution (Bhowmik et al., 2022).

Fisheries occupy an important position in Bangladesh's economy. And due to the development of the country's fishing industry in recent years, the harvest of fish in Bangladesh has been increasing year by year. According to Shamsuzzaman et al. (2017), the total yields of Bangladeshi inland and marine fisheries were 1401560 t and 379497 t throughout 2000 to 2001, and the yields increased to 3048399 t and 599846 t throughout 2014-2015. Correspondingly, with the development of aquaculture, dried fish as a one of the major products of the fresh fish industry has developed dramatically. From year 2018 to 2019, the exported amount of dried fish was 3144 tons, which is about 10 times higher than that in 2008. These exports of dried fish brought Bangladesh about 52 million CA dollar of foreign currency (Kubra et al., 2020). In addition, the dried fish industry has also alleviated the employment pressure of some people in the coastal region, especially women because they play a major role in processing, transportation and marketing of the products (Paul et al., 2018).

Dried fish (DF) is a common value-added fish product that can be stored at room temperature without the requirement for additional packaging (Belton et al., 2022). Depending on the method of packaging and preservation conditions, DF can be kept for 3-6 months at

room temperature (Immaculate et al., 2012; Uddin et al., 2017). As described by Albaris Tahiluddin & Kadak (2022), salting, drying, smoking, pickling/marinating, and fermentation are well recognized as traditional fish processing techniques that have been widely applied worldwide, especially in Asia. Taking one of the most used practices, sun-drying, as an example, and depending on the size of the fish, pre-treatments such as grading, dressing, descaling, washing, salting, etc. may or may not be performed (Paul et al., 2018). The drying process usually takes hours to days, depending on the weather and the condition of the fish's surface area (Paul et al., 2018).

DF is well valued for the condensed protein content, thus representing an excellent alternative to fresh fish in both inland and coastal areas, as DF is convenient to consume and transport, it is relatively affordable, and not limited by season. In addition to the rich protein content, multiple studies have reported DFs contain condensed levels of unsaturated fatty acids, especially n-3 fatty acids, which have been proven to have beneficial effects on the human body. Most importantly, with facing greater challenges of malnutrition world-wide, especially in developing countries, DFs have been reported to contain multiple minerals (iron, zinc, potassium, sodium, calcium etc.) that can be used as a potentially effective and practical mitigation method to reduce the incidence of malnutrition (Bhowmik et al., 2022). Thereby, there has been an increased experimental interest in studying the nutritional aspects of DF in recent years (Belton et al., 2022).

However, previous in-depth research activities on the chemical and nutritional composition of DFs had revealed some concerns. These include contaminants, which may be introduced to DFs via extensive processing techniques (breeding of harmful microorganisms,

mosquitoes and flies), and deliberately adding harmful pesticides (to avoid the above-mentioned problems during the production, trade, and retail processes). Therefore, there is the need for scientists to pay more attention on monitoring the safety and quality of DFs. What's more, water body pollution can consequently transfer the pollutants into the fishes that live in the environment. It has been reported that DFs may also be under the risk of excessive heavy metals and microplastic contamination, which may cause chronic diseases (such as anemia and cancer) and other unpredictable harms to the human body (Ghosh et al., 2021; Hossain et al., 2017; Mansur et al., 2013). In addition, high concentrations of biogenic amines and their possible health effects, especially in DFs that have been stored for long periods of time, are also challenging the health and safety of consumers (Amascual et al., 2020).

As reported by the Food and Agricultural Organization (FAO) in 2018, changes in the climatic environment will cause unemployment of the population directly engaged in fisheries. Therefore, finding alternative employment opportunities is a solution to alleviate poverty and ensure employment (FAO, 2018). Out of the aim to increase the economic benefit and minimize wastes, interests have been focused on DF protein extraction and their functional properties with respect to food product development or fortification (Sarkardei & Howell, 2007). DF major nutrients are the proteins, which have a wide range of applications in food systems as functional food additives (Mazorra-Manzano et al., 2017). For example, it has been reported that modified proteins can be used as emulsifiers in various food systems including salad dressings (Lee et al., 2006). Surimi made from pomfret meat can form a gel with high elasticity, viscosity, and rigidity, so it can be used as a binding material for other food products (Lou et al., 2000; Lou, Wang, et al., 2000). Increased value addition of fish is likely to also benefit

fishermen and downstream industries. Meanwhile, exploring the functional properties of DF proteins also lays the foundation for developing innovative foods in the future.

1.1 Hypotheses

a) Different species of DF will have different protein contents, water content, lipid content, ash content, mineral profile according to variations in the production process and location.

b) DFs will be a good source of vitamin B_{12}, and the content may vary depending on the origin, species and production procedure of the sample.

c) DFs may be contaminated by heavy metals such as chromium (Cr), arsenic (As), mercury (Hg), and lead (Pb). The content and type of the heavy metal may vary according to the origin, species, and production procedure of the sample.

d) DFs will also contain excessive amount of iron (Fe), copper (Cu), zinc (Zn).

e) The free amino acid content will be elevated in some or all samples due to protein degradation during drying.

f) DF protein isolates will have altered protein structural properties and functionalities including solubility, emulsification, foaming, gelation, water and oil holding capacity, heat coagulability.

1.2 Objectives

The overall aims of this research are to determine the nutritional value of DFs, their potential health risks from consumption and the functionally relevant properties of the isolated proteins.

Therefore, the specific objectives of the proposed study are to:

a) Determine the proximate composition and mineral profiles of DFs obtained by different processing methods and from different locations in Bangladesh.

b) Determine lipid, cholesterol, amino acid, free amino acids, and heavy metal profiles of the DFs.

c) Determine the digestibility of the DFs.

d) Use isoelectric protein precipitation method to produce dried fish protein isolates (DFPIs).

e) Determine the structural and functional properties of the DFPIs

CHAPTER TWO

2 LITERATURE REVIEW

2.1 Nutritional studies of DFs

This section reports on some of the existing research on the proximate composition, minerals profile, fatty acid composition, amino acid profile, and vitamin contents of DFs.

2.1.1. Proximate analysis of DFs

Table 1 summarizes nine research works on 32 species of DF and their proximate composition data. Different dried fish species as well as drying methods will affect the proximate composition. Compared with other drying methods, the moisture content of sun-dried fish is relatively high, most of which ranged 20-30%. However, exceptions such as *T. haumela* with 14.53% (Al Banna et al., 2022) and *C. ranga* with 15.66% (Rana et al., 2020) contained less moisture. Other drying methods such as smoke, solar, curing (salt), and freeze-drying, decreased the moisture content in DF to a range of <15%. Among the drying methods, the freeze-drying reported by (Sablani et al., 2001) produced the lowest moisture content of 4.94% in *S. longiceps*. Generally, the protein content of DFs fluctuates between ~30-70% (dry weight basis, dwb) and specifically that of sun-dried DF ranges from ~45-68% (dwb). It was found that the protein content of H. nehereus is relatively high among all species (sun-dried: average 56.29% and solar-dried: 68.78%), which partly explains why it appears repeatedly in many previous reports. In addition, it was found that the protein content of solar dried and freeze-dried DFs was relatively high, with freeze-dried *S. longiceps* and solar dried *B. bayad* containing 71% and 82% protein contents, respectively (Olokor & Ngwu, 2009; Sablani et al., 2001). The ash content of DFs ranged from 6 to 20%, with the highest level (20.78%) detected in plant extracts treated and salted *O. niloticu* (Dharmadasa et al., 2019). In terms of lipid content,

drastic differences were observed among varied species; sun-dried *H. walga* contained as high as 26.13% (Azam et al., 2003), while sun-dried *H. nehereus* contained 3.5% and ranked the lowest (Azam et al., 2003).

Table 1. Proximate composition of dried fishes

Species	Treatment	Moisture (%)	Protein (%)	Ash (%)	Lipid (%)	Carbohydrate (%)	References
M. cephalus	Sun-dried	19.93	68.09	7.45	4.87	nd	Azam et al. (2003)
S. sorrakowah		23.49	58.35	11.32	7.84	nd	
S. phasa		24.46	62.36	9.51	3.67	nd	
H. nehereus		21.26	61.25	15.02	3.5	nd	
A. caelatus		19.22	66.52	5.08	9.03	nd	
H. ilisha		23.9	40.69	9.11	26.13	nd	
P. paradiseus		21.65	57.25	12.14	8.95	nd	
T. haumella		23.61	53.85	10.78	11.71	nd	
P. chinenchis		18.23	63.46	10.91	7.1	nd	
H. walga		21.08	54.19	11.01	25.3	nd	
M. hagio		20.98	56.77	9.98	11.19	nd	
E. lanceolatus	Sun-dried	23.19	61.24	6.32	7.94	nd	Azam et al. (2003)
C. bengalensis		21.7	54.86	11.85	11.44	nd	
T. patoka		23.31	57.51	7.22	9.69	nd	
H. nehereus	Sun-dried	21.65	52.06	20.07	5.38	nd	Al Banna et al. (2022)
T. haumela		14.53	62.63	14.87	7.27	nd	
C. mrigala	Cured	8.5	62.8	-	9.6	nd	Pradhan et al. (2018)
S. longiceps	Freeze-dried	4.94	71	15	10	nd	Sablani et al. (2001)

Table 1. - *contd.*

Species	Treatment	Moisture (%)	Protein (%)	Ash (%)	Lipid (%)	Carbohydrate (%)	References
H. nehereus	Solar tunnel dried	15.25	68.78	8.29	7.37	nd	Haque et al. (2013)
P. argenteus		13.43	68.05	9.45	8.51	nd	
O. niloticu	Plant extracts treated + salted dried	9.85	29.02	20.78	8.31	24.76	Dharmadasa et al. (2019)
C. gariepinus	Smoked	7.3	68.4	6.4	12.5	1.8	Foline et al. (2011)
B. bayad	Solar dried	10.6	82.14	-	8.46	0.4	Olokor & Ngwu (2009)
A. mola	Sun-dried	24.85	48.94	16.12	10.06	nd	Rana et al., 2020
G. chapra		27.32	49.6	14.31	8.76	nd	
P. atherinoides		27.05	45.8	15	11.8	nd	
P. sophore		25.13	48.82	16.11	9.84	nd	
M. tengara		27.52	43.2	15.22	12.84	nd	
C. soborna		28.2	49.84	12.63	9.15	nd	
C. punctatus		35.5	42.06	18.24	4.2	nd	
H. nehereus		28.96	55.56	7.96	8.06	nd	
C. ranga		15.66	50.53	18.6	13.03	nd	

2.1.2. Mineral profile of DF

Table 2 summarizes four species of DF and their detailed mineral profiles. DFs contain a variety of minerals including magnesium (Mg), iron (Fe), copper (Cu), zinc (Zn), phosphorus (P), calcium (Ca), sodium (Na), and potassium (K). In general, the mineral content of DFs is affected by the species, for example, the iron content detected in *O. niloticus* is 2-3 times that of the DFs reported in Bhowmik et al. (2022) while the calcium content of *G. chapra* is 1.5 times higher than the levels in *C. soborna* and *P. ticto* (Bhowmik et al., 2022). In addition,

selected DF parts will also affect the mineral profile of the DF, as the mineral content in fish bones, scales, teeth, and heads are significantly different from the muscle. Bhowmik et al. (2022) reported that the calcium content of whole *G. chapra* powder (5190.9 mg/100 g) was significantly higher than that in the muscle (2509.14 mg/100 g) while the potassium content in *P. ticto* muscle powder (851.21 mg/100 g) is one-third higher than that of the whole *P. ticto* powder (621.84 mg/100 g).

In general, DFs are considered as good sources of various minerals. Among the elements reported in Table 2, iron is the most insufficiently consumed nutrient globally, as its deficiency is related to the onset of anemia, which affects almost one-third of the world's population (Fairweather-Tait & Sharp, 2021). According to the Food and Nutrition Board & Institute of Medicine (2001b), the recommended dietary allowance (RDA) of iron is 27 mg/d (14–30-year-old pregnant women). Assuming that the daily intake of DF is 15 g, sun-dried *C. soborna* with the lowest iron content can meet roughly 12% of the RDA. Appropriate calcium intake can reduce gestational hypertension, lower blood pressure (especially in young people), prevent colorectal adenomas, and lower cholesterol levels, which is also the key to the healthy growth and proper functioning of bones and teeth (Cormick & Belizán, 2019). A reference value of 1300 mg/d for the 14-18 year-old population was proposed by Institute of Medicine (US) Standing Committee (1997) indicating that when consuming 15 g of DF per day, this group of people will no longer be at risk of calcium deficiency. Zinc deficiency is commonly reported in South and Southeast Asia, sub-Saharan Africa, and Central America, with approximately 17.0-29.6% of the population at risk of inadequate intake (Gupta et al., 2020). Zinc deficiency is associated with perioral dermatitis, alopecia, diarrhea, impaired wound healing, dysgeusia, immune

deficiency, and increased incidence of bacterial, fungal, and viral infections, which in children can lead to malnutrition and even death (Chasapis et al., 2020). The RDA of zinc (Food and Nutrition Board & Institute of Medicine, 2001c) is 13 mg/d (14–18-year-old and lactating women). Therefore, consumption of 15 g of DFs can support the body with about 13.8% zinc RDA (Bhowmik et al., 2022). Copper deficiency can affect bone marrow hematopoiesis and the normal function of the nervous system and can also cause anemia (Myint et al., 2018). Bhowmik et al. (2022) reported that the copper content in *P. ticto* powder was 0.53 mg/100 g, suggesting that supplementation through DF consumption may be feasible. As the RDA of copper is 1.3 mg/d for pregnant women aged 14–30-years (Food and Nutrition Board & Institute of Medicine, 2001a), then 15 g of *P. ticto* powder can meet 4.6% of the copper RDA.

Table 2. Mineral composition of dried fishes (mg/100 g)

Species	Treatment	Mg	Ca	Fe	P	Na	K	Zn	Cu	References
O. niloticus	60 ℃ oven dried	nd	nd	89	398	nd	154	nd	nd	Emmanuel et al. (2020)
G. chapra	Sun-dried ready-to-use fish powder	188.28	5190.9	43.7	2604.1	107.77	268.64	12	0.45	Bhowmik et al. (2022)
C. soborna		185.63	3597	21.6	2132.54	198.42	518.88	12.83	0.4	
P. ticto		149.89	3891.56	32.8	2092.22	206.05	221.03	12.74	0.53	

2.1.3. Essential amino acids (EAA) profile of DF

Table 3 summarizes seven species of DFs for their EAA (histidine, threonine, valine, methionine, isoleucine, leucine, phenylalanine, tryptophan, and lysine) profile. Due to the absence of metabolic pathways required for the synthesis of essential amino acids in the human body, an external supply from the diet is required (Lopez & Mohiuddin, 2023). The total EAA content fluctuates in the range of 10-46 g/100 g sample, with most of the DFs containing more than 20 g/100 g. *P. ticto*, *C. cyanopterus*, and *S. barracuda* were reported with the highest EAA contents of 46.0, 38.3, and 39.3 g/100 g, respectively (Bhowmik et al., 2022; Iko Afé et al., 2021). According to the referenced requirement of each EAA, the DFs can be considered as good EAA sources except the *C. soborna* and *G. chapra*. In addition, the EAA contents of *C. cyanopterus* and *S. barracuda*, except tryptophan, were much higher than the reference requirement.

Table 3. Essential amino acid profile (g/100 g of sample)

Species	Treatment	His	Thr	Val	Met	Ile	Leu	Phe	Trp	Lys	Total EAA	References
G. chapra	Sun-dried ready-to-use fish powder	nd	3	1.4	0.3	0.9	1.2	0.6	1	1.6	10.8	Bhowmik et al. (2022)
P. ticto		2	5.3	4.6	2.1	4.7	7.4	2.9	3.9	7.4	46	
C. soborna		0.6	2.6	1.7	0.8	1	1.7	1	1.5	2.9	17.7	
C. [illegible]	Smoke dried	2.9	3.8	4	2.4	3.5	6.4	3.2	nd	7	38.3	Iko Afé et al. (2021)
E. fimbriata		1.7	2.8	3	1.9	2.6	4.8	2.6	nd	5.3	28.6	
S. barracuda		1.7	3.9	4.2	2.7	3.7	6.8	3.5	nd	7.5	39.3	
Grass carp	Semi-dried	nd	3.2	3.9	2.8	3.6	5	2.5	nd	4.2	29.7	Liu et al. (2022)
Amino acid requirements		1	1.5	2.6	1.5	2	3.9	2.5	4	3	nd	FAO Expert Consultation (2011)

His: Histidine; Thr: Threonine; Val: Valine; Met: Methionine; Ile: Isoleucine; Leu: Leucine; Phe: Phenylalanine; Trp: Tryptophan; Lys: Lysine; EAA: essential amino acid

2.1.4. Fatty acid composition of DF

Table 4 includes the fatty acid profile of five selected DFs and their processing methods. In general, DF contains varied levels of saturated fatty acid (SFA, 25-50 g/100 g lipid), monounsaturated fatty acid (MUFA: 21.9-39.12 g/100 g lipid), polyunsaturated fatty acid (PUFA: 15.40-34.74 g/100 g lipid), with values dependent on the species and processing methods, which is consistent with previous reports (Ogunbambo, 2020; Slavin et al., 2016; Sroy et al., 2023; Tenyang et al., 2020). A higher level of SFA was detected in drum dried and sun-dried DFs compared with the eco-friendly dried and smoke-dried DFs (Ogunbambo, 2020; Tenyang et al., 2020). In addition, under the same processing treatment, a higher level of PUFA was detected in *L. falcipins* (sun-dried: 27.05 g/100 g lipid, smoke-dried: 22.23 g/100 g lipid) compared that of *O. niloticus* (sun-dried: 22.23 g/100 g lipid, smoke-dried: 18.93 g/100 g lipid).

An adequate consumption (dietary intake 0. 25-0.5 g/day) of eicosapentaenoic acid (EPA) and docosahexanoic acid (DHA) was recommended by the European Food Safety Authority (EFSA, 2010), for their health benefits towards cardiovascular diseases prevention. Islam et al. (2021) suggested that consumption of EPA and DHA can positively affect formation of the nervous system and reduce liver steatosis. *C. gariepinus* have shown its ability to serve as a good source of DHA and EPA (Ogunbambo, 2020). In addition to the contents of individual n-3 fatty acid, DHA and EPA, the ratio of total n-3/total n-6 fatty acid is another crucial indicator of a food lipid quality. As reported by Coskuntuna et al. (2015), values below 0.25 n-3/n-6 ratio may promote cardiovascular disease due to the negative health risks associated with excessive levels of dietary n-6 fatty acids. In the sense of screening for a better source of fat, consumption of DFs selected in this review should at least not increase the risk of cardiovascular disease.

Wood et al. (2008) reported that a PUFA/SFA ratio higher than 0.4 in diet will benefit health and a ratio lower than 0.38 may promote the onset of obesity (Phillips et al., 2012). DFs selected in this review, except from *H. siamensis*, all show high values of the PUFA/SAF ratio, and thus can be used to supplement diets that are low or lacking in PUFA.

Table 4. Fatty acid profile of dried fishes (g/100 g lipid)

Species	Treatment	Total SFA	Total MUFA	Total PUFA	EPA	DHA	n-3/n-6 ratio	PUFA/SAF ratio	References
C. gariepinus	Drum dried	30.2	36.49	33.32	7.8	16.2	2.62	1.1	Ogunbambo (2020)
	Eco-Friendly	25.11	37.04	34.74	6.77	17.26	2.26	1.38	
H. siamensis	50°C oven dried	50	21.9	15.4	2.6	3.2	2.76	0.31	Sroy et al. (2023)
L. falcipins	Sun-dried	39.53	32.78	27.05	0.26	2.83	0.97	0.68	Tenyang et al. (2020)
	Smoke-dried	32.54	35.69	22.23	0.19	2.11	0.89	0.68	
O. Niloticus	Sun-dried	42.82	36.94	22.37	1.19	1.75	0.32	0.52	
	Smoke-dried	36.23	39.12	18.93	0.77	1.66	0.5	0.52	
R. argentea	Spice treated 75°C oven dry	39.12	34.12	26.76	6.72	7.68	3.27	0.68	Slavin et al. (2016)

SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; EPA: eicosapentaenoic acid (n-3, C20:5); DHA: docosahexanoic acid (n-3, C22:6n3)

2.1.5. Vitamin content of DF

DFs contain various vitamins, ranging from the water-soluble to the lipid-soluble. Alahmad et al. (2021) reported that oven-dried *H. nobilis* contained vitamins B_1, B_2, B6, B_{12}, C and D_3 at 0.1, 0.24, 0.59, 0.01, 1.07, and 0.01 g/100 g dry matter (DM), respectively. Sroy et al. (2023) reported that 50°C oven-dried *H. siamensis* contained 79.1 μg/100 g DM of vitamin A. Chukwu (2009) and Ogbonnaya & Shaba (2009) reported 530 and 460 μg/100 g DM of vitamin A, 1 and 4 g/100 g DM of vitamin C in kiln-dried *C. gariepinus* and *O. nilotieus*. Scott & Latshaw (1994) reported 0.006 and 0.003 g/100 g DM of vitamin D_3 in 25°C-freeze-dried and 25°C-oven-dried *B. tyrannus*.

Vitamin A is an essential nutrient that maintains the immune system, eye health, while its deficiency is common among pre-school children and pregnant women (Rice et al., 2004). Vitamin A dissolves in fat and is vulnerable to oxygen and high temperature-induced degradation. Sroy et al. (2023) reported that high oven drying temperature treatments (50, 60, 70, 80℃) of *H. siamensis* decreased the vitamin A content significantly (raw material contained 1698.5 μg/100 g DM while the 80℃ oven dried had only 85.9 μg/100 g DM). Chukwu (2009) and Ogbonnaya & Shaba (2009) reported that electric drying seems to be better than kiln dry in preserving vitamin A, as the contents in electric-dried *C. gariepinus* and *O. nilotieus* were higher than those of the kiln-dried products. According to Penniston & Tanumihardjo (2003), for pregnant women aged >18 years, their vitamin A RDA is 1300 μg/d. In order to alleviate the consequent health hazards cause by vitamin A deficiency, *C. gariepinus* and *O. nilotieus* produced from both electric drying and kiln drying (Chukwu, 2009; Ogbonnaya & Shaba, 2009) can serve as good dietary supplements.

2.2 Safety issues with DF consumption

Due to the drying process, moisture loss is usually significant, thereby concentrating the nutrients in DFs. However, contaminants such as heavy metals and microplastics that naturally occur in fishes also become concentrated in the DFs (Sroy et al., 2023). In addition, DFs are vulnerable to mosquito and bacteria breeding, which could introduce pathogenic microorganisms. Therefore, merchants may deliberately add pesticides to DFs to avoid the above problems, which inevitably leads to contamination (Belton et al., 2022). In this section, the heavy metal, microplastic, and pesticide contents of DFs are discussed. However, this thesis report includes only heavy metal research.

2.2.1. Heavy metal

Table 5 summarizes 6 studies on 22 species of DFs for their heavy metal profiles. In general, among the selected DFs, the content of arsenic (As) and cadmium (Cd) were below the referenced allowance values, thereby not at risk of toxicity. The most excessive case was observed in chromium (Cr), as some of the DFs were detected to contain excessive levels, which reached 12.4 mg/kg DM in *G. chapra* (Rakib et al., 2021). The mercury (Hg) content in DFs ranged 0.08-60.2 mg/kg DM and ranked the second most excessive with the highest level found in *I. megaloptera* (Rakib et al., 2021). Excessive lead (Pb) levels were not common in the DFs, except for *H. nehereus* and *T. haumela* that contained 8.281 and 5.465 mg/kg DM, respectively (Hossain et al., 2017).

As reported in numerous studies, consuming food products that are contaminated with heavy metals triggers adverse effects on human health. For example, Hg is associated with neurological damages, Cd causes carcinogenic diseases, Pb is a neurotoxin that causes

behavioral deficits in vertebrates and may lead to decreased survival, growth rate, and learning ability while chromium has carcinogenic and ulcerative properties (Rakib et al., 2021). In addition, even though copper (Cu), zinc (Zn), and iron (Fe) are crucial in the normal functioning of several physiological processes of the human body, their excessive intakes may also cause negative consequences. Rakib et al. (2021) suggested that excessive levels of Cu in the human body is associated with anemia, Zn overdose can cause lung disease, gastroenteritis, fever, vomiting, muscle coordination problems, and dehydration while Fe levels beyond recommended amounts can result in neurological and psychological disorders (Rakib et al., 2021).

Therefore, to prevent the risk of anemia, cancer, and other chronic diseases caused by excessive intake of heavy metals, the daily recommended intake of dried fish should be carefully determined. This intake should consider not only that DFs may be the main source of animal protein in some areas, but also the potential harm that could be caused by heavy metals to the human body after excessive intakes of such foods.

Table 5. Heavy metal profile of dried fishes (mg/kg Dry Matter)

Species	Treatment	Arsenic (As)	Cadmium (Cd)	Chromium (Cr)	Lead (Pb)	Mercury (Hg)	References
H. nehereus	Sun-dried	nd	0.824	8.380	8.281	nd	Hossain et al. (2017)
T. haumela	Sun-dried	nd	0.485	6.969	5.465	nd	
H. nehereus	Sun-dried	nd	0.427	0.282	0.050	nd	Hoque et al. (2022)
T. haumela	Sun-dried	nd	nd	0.431	0.078	0.080	
H. neherius	Sun-dried	<0.41	nd	7.060	0.520	28.700	Rakib et al. (2021)
T. lepturus	Sun-dried	<0.41	nd	9.340	0.280	48.300	
P. chinensis	Sun-dried	<0.41	nd	0.420	0.001	28.500	

Table 5. - *contd.*

Species	Treatment	Arsenic (As)	Cadmium (Cd)	Chromium (Cr)	Lead (Pb)	Mercury (Hg)	References
P. affinis	Sun-dried	<0.41	nd	3.550	0.001	43.600	Rakib et al. (2021)
A. mola	Sun-dried	<0.41	nd	5.460	0.001	30.300	
P. microdon	Sun-dried	<0.41	nd	6.670	0.001	38.700	
I. megaloptera	Sun-dried	<0.41	nd	9.350	0.001	60.200	
C. dussumieri	Sun-dried	<0.41	nd	7.790	0.001	37.300	
L. calcarifer	Sun-dried	<0.41	nd	7.390	0.001	26.500	
G. chapra	Sun-dried	<0.41	nd	12.400	0.001	36.500	
H. nehereus	Sun-dried	nd	0.029	0.323	nd	nd	Akter et al. (2019)
A. mola	Sun-dried	nd	0.018	0.307	nd	nd	
D. devario	Sun-dried	nd	0.046	0.742	nd	nd	
C. chandramara	Sun-dried	nd	0.055	0.366	nd	nd	Akter et al. (2019)
N. vigatus	Sun-dried	nd	0.021	0.370	0.257	nd	
T. lepturus	Sun-dried	nd	0.016	0.550	nd	nd	
C. striatus	Sun-dried	0.003	0.089	0.045	nd	nd	Mansur et al. (2013)
L. rohita	Sun-dried	0.003	0.053	0.025	nd	nd	
W. attu	Sun-dried	0.003	0.097	0.068	nd	nd	
P. pangasius	Sun-dried	<0.41	nd	5.440	0.480	1.070	Ghosh et al. (2021)
O. niloticus	Sun-dried	<0.41	nd	2.260	0.340	0.790	
H. fossilis	Sun-dried	<0.41	nd	4.180	0.450	0.570	
A. testudineus	Sun-dried	<0.41	nd	6.730	0.350	0.740	
C. batrachus	Sun-dried	<0.41	nd	<0.41	0.490	0.880	
Referenced allowance value		1.4	1	0.15	0.5	0.5	Ghosh et al. (2021) and Hossain et al. (2017)

2.2.2. Pesticides

Table 6 summarizes the contents of dichlorodiphenyltrichloroethane (DDT), chlorpyrifos and cypermethrins in 12 species of DFs. DDT is one of the most common pesticides used in dried marine fish and is banned in 49 countries due to potential long-term health effects (Kar et al., 2020). The use of chlorpyrifos in food was banned in 2000, and any detection of this chemical is considered adulteration (Ofuani & Destiny, 2022). Prolonged exposure to cypermethrin can cause chronic and persistent neurotoxicity/neurological effects, abortion, teratogenic effects, and immunosuppression (Ullah et al., 2018). Ofuani & Destiny (2022) reported that smoke-dried *C. gariepinus* and *E. fimbriat* contained 1.232 and 0.727 mg/kg DM of DDT, and 0.054 and 0.042 mg/kg DM of chlorpyrifos respectively, which contents were higher than the maximum residue limit (MRL) of 0.01 mg/kg DM for both compounds as set by the FAO (FAO & WHO, 2023). Maïworé et al. (2021) reported excessive contents of chlorpyrifos and cypermethrins in multiple species of DFs including *C. laticeps*, *P. bovei bovei*, *M. elongatus*, *P. annectens brieni*, *T. dageti*, and *M. senegalensis* with the highest level of chlorpyrifos found in *P. bovei bovei* (8.8 mg/kg DM) and highest level of cypermethrins in *T. dageti* (15 mg/kg DM). Kar et al. (2020) reported that the DDT content in *P. chinensis*, *H. nehereus*, and *T. savala* was below the MRL of 0.01 mg/kg DM.

Table 6. Pesticide residues content of dried fishes (mg/kg Dry Matter)

Species	Treatment	DDT	Chlorpyrifos	Cypermethrins	References
P. chinensis	Sun-dried	<0.01	nd	nd	Kar et al. (2020)
H. nehereus	Sun-dried	<0.01	nd	nd	
T. savala	Sun-dried	<0.01	nd	nd	
C. laticeps	Dried	Nd	nd	0.520	Maïworé et al. (2021)
P. bovei bovei	Dried	Nd	8.800	0.300	
M. elongatus	Dried	Nd	0.190	3.600	
P. annectens brieni	Dried	Nd	2.900	0.120	
T. dageti	Dried	Nd	nd	15.000	
G. niloticus	Dried	Nd	nd	0.015	
M. senegalensis	Dried	Nd	0.035	0.660	
C.gariepinus	Smoke-dried	1.232	0.054	nd	Ofuani & Destiny (2022)
E.fimbriata	Smoke-dried	0.727	0.042	nd	
Maximum residue limit (MRL) level		0.01	0.01	0.02	(FAO & WHO (2023)

2.2.3. Microplastics (MPs)

Microplastics (MPs) have been detected in DF products around the world and thereby may pose a serious threat to consumer health. Hasan et al. (2023) reported that the MPs contents in dried *H. nehereus*, *T. lepturus* and *S. phasa* collected from Bangladesh were higher than those in fresh fish, with the MPs characterized by filamentous (66%), <500-μm size (39.66%) and composed of low-density polyethylene (LDPE, 38%), polystyrene (PS, 22%), polyvinyl chloride (PVC, 16%), and polyamide (PA, 13%). Rukmangada et al. (2023) reported that 21 different species of DFs in India were positive for MPs, which was characterized by fragmented, <100-μm sized (47%) polypropylene (56%), LDPE (17.5%), and PS (15.5%). Kutralam-Muniasamy et al. (2023) found MPs in dried *C. jordani* (collected in Mexico) were

characterized by their <500-μm size (84%) filamentous structure, and were composed of polyester, acrylonitrile butadiene styrene, polyvinyl alcohol, ethylene-propylene copolymer, nylon 6 (3), cellophane and viscose. In addition, the authors emphasized that the abundance of MPs detected in dried *C. jordani* was higher than that of the previously reported values. Karami et al. (2017) detected higher levels of MPs in dried *C. subviridis* and *J. belangerii* muscles than that of the whole DFs, suggesting that evisceration may not reduce the risk of MPs contamination in consumers' diets. Therefore, facing the new challenges posed by MPs to food safety, more research is needed to determine the potential human health effects of their presence in DFs.

2.3 Dried fish protein isolates (DFPIs)

This section will include the structural characteristics of DFPIs (myofibrillar protein, salt-soluble protein, sarcoplasmic protein and gelatin), including information on their secondary structure, molecular weight, chemical bond composition shift, and surface hydrophobicity, as well as their functional properties such as solubility (and consequent impact on extraction yield), least gelation concentration, gel strength, emulsifying properties, foaming properties, and water retention capacity.

2.3.1. Structural characteristics of DFPIs

Drying and concomitant or pretreatment heating process can induce denaturation of proteins (myofibrillar proteins, sarcoplasmic proteins, salt-soluble proteins and gelatin), which has been discussed in several reports (Irshad et al., 2023; Jiao et al., 2022; Nie et al., 2022; Shaviklo et al., 2012). The most apparent phenomenon reflecting protein denaturation is the contraction of muscle structure after water loss. As reported by Jiao et al. (2022), in the

histological study of muscle of hot-air dried *P. crocea*, the myofibrils were incoherent and severely disordered accompanied with significantly expanded extracellular space. Chen et al. (2022) also reported a similar phenomenon of breakage, contraction, condensing, and hardening of muscle fiber in hot-air dried and heat-pump dried *T. ovatus*. The significantly increased surface hydrophobicity also indicates that the protein is denatured during drying and heating, as the high temperatures used in these processes destroy the native structure of proteins, which consequently result in the exposure of previously hidden internal hydrophobic groups. Jiao et al. (2022), Chen et al. (2022), and Niu et al. (2019) have reported significant increases in surface hydrophobicity of myofibrillar proteins treated by hot air drying (HAD), heat pump drying (HPD), vacuum freeze drying (VFD) and vacuum spray drying (VSD).

Denaturation of DFPIs can be seen as a result of shift in the chemical bond (and intermolecular force) composition of the protein during the drying process and its corresponding changes in the structural components. Raghunath et al. (1995) reported that the total sulfhydryl group (SH) content decreased regularly and sharply with the increase of drying temperature during a 24 h drying process (50℃: 13.62 μmol/g DM; 60℃: 4.76 μmol/g DM; and 70℃: 3.50 μmol/g DM) in *N. japonicus*. In addition, prolonged drying duration also decreased the sulfhydryl group content significantly with values of 27.30 μmol/g DM at the 0 h, which dropped to 13.62 μmol/g DM after 24 h of 50℃ drying (similar trend was observed in 60℃- and 70℃-treated samples). The decreases in sulfhydryl contents suggest that this type of chemical group is vulnerable to temperature as well as drying and are involved in the formation of new chemical bounds (Raghunath et al.,1995). Therefore, it is proposed that formation of new disulfide bonds (-S-S-) may involve the sulfhydryl groups. Odoli et al. (2019)

observed approximately 50% and 17% increases in disulfide bond contents in tunnel-dried + blanched and tunnel-dried + salted *M. villosus* salt-soluble protein, accompanied by drastic decreases in the contents of sulfhydryl group.

As the denaturation of DFPIs cause perturbation of non-covalent interactions (e.g. hydrogen bonds, electrostatic interactions, and hydrophobic interactions), changes in their secondary structure components (α helices, β turns, β sheets and random coils) should be expected. Chen et al. (2022) reported that HAD, HPD, and FD *T. ovatus* myofibrillar proteins showed a downward trend in α-helix and β-sheet contents, as well as an upward trend in β-turn and random coil. Similar trends were also observed by Niu et al. (2019), Sarkardei & Howell (2007), and Nie et al. (2022) This transition of protein-secondary structure from stable (α-helices and β-sheets) to unstable (β-turns and random coils) implies an increase in protein-protein interactions, formation of macromolecular aggregates, with resultant changes in protein functional properties (Chen et al., 2022).

The impact of drying, temperature, and salting on proteins will directly reflect on the molecular weight (MW) of the polypeptides present in the proteins. The main characteristic bands of myofibrillar proteins are myosin heavy chain (MHC) of ~200 kDa and actin of 50 kDa (Hashimoto et al., 2004). Chen et al. (2022) reported the disappearance of the MHC band and the appearance of some small MW (16 kDa) bands in HAD *T. ovatus*, indicating that heat treatment causes the degradation of myosin to form smaller size polypeptides, accompanied by formation of high molecular weight macromolecular protein aggregates that cannot enter the separation gels. Shaviklo et al. (2012) also reported the disappearance of the MHC band in FD *P. virens*. Raghunath et al. (1995) proposed that the weakening of MHC band is related to the

decrease in stainability of Coomassie brilliant blue caused by protein denaturation. Nie et al. (2022) reported a weakened (varying degrees) intensity of the characteristic bands of collagen (β chain: 280 and 260 kDa, α1 chain: 140 kDa, and α2 chain: 125 kDa) in prolonged freeze-dried tilapia skin.

2.3.2. Functional properties of DFPIs

The denaturation of proteins is inseparable from the changes in chemical bonds and intermolecular forces, which not only affect the structural characteristics of proteins, but also their functional properties (including solubility, emulsification, gelling, foaming and water-holding properties).

In general, factors such as drying, salting, and temperature will significantly reduce the solubility of proteins (Chen et al., 2022). As mentioned above, denaturation leads to the exposure of hydrophobic groups, resulting in hydrophobic-induced protein aggregation (Niu et al., 2019). Denaturation also induces oxidation of the sulfhydryl group to form the disulfide group, resulting in cross-linking of the protein chains (Odoli et al., 2019). In addition, there is protein condensation, which facilitates formation of disulfide bonds (Hashimoto et al., 2004), causes soluble protein content to decrease, and results in decreased extractability and yield of proteins. For example, Sarkardei & Howell (2007) reported a significantly 80% decrease in the protein yield of FD *S. scombrus* and *T. trachurus*.

Shaviklo et al. (2012) reported that the gel-forming ability (or least gelation concentration) of DF myofibrillar protein isolated from *P. virens* was worse than that of surimi (surimi: 6% protein, DFPIs: >10% protein). The author (Shaviklo et al., 2012) discussed that the gel-forming ability was affected by the degree of myofibrillar protein denaturation, the relative

concentration of myofibrillar/sarcoplasmic protein ratio, and the appearance of additives (to protect the proteins during frozen storage and drying). However, Giménez et al. (2005) reported that although there were differences in MW patterns between salted-dried *S. Vulgaris* skin gelatin and standard gelatin, it did not lead to obvious changes in gel strength, indicating that the presence of a large amount of high molecular weight polymers in the gelatin preparation can produce similar gel strength values.

Niu et al. (2019) reported that vacuum freeze-drying and vacuum spray drying treatments increased the emulsion stability of *H. molitrix* myofibrillar protein by 2-fold and 8-fold, respectively, which was attributed to molecular flexibility of the treated proteins (lower α-helix percentage and increased surface hydrophobicity). Increased emulsion stability due to drying was also found in freeze-dried tilapia skin gelatin (Nie et al., 2022).

In the freeze-dried myofibrillar protein of *P. virens*, a higher foaming capacity but lower foam stability was reported than that of surimi, indicating that there is no necessary connection the two foam properties (Shaviklo et al., 2012). Instead, the foaming capacity of DFPIs is affected by protein concentration, pH, salt, sugar, lipids, and foam formation method (Shaviklo et al., 2012).

A decrease in water holding capacity (from 6.4 g water/g protein to 4.2 g water/g protein) was reported in vacuum freeze-dried *H. molitrix* myofibrillar protein, presumably related to the loss of soluble protein and reduced availability of polar amino acids in the sample (Niu et al., 2019). In contrast, in the same study, an increased water holding capacity was reported in vacuum spray-dried samples, possibly due to the exposure of some functional groups that were previously buried inside, resulting from an increase in the protein surface area.

2.4 Conclusions

Although the existing research fills in some of the critical experimental data on the proximate chemical composition, heavy metal content, and vitamin content of some dried fish. However, it should be noted that dried fish is a complex collective term. Differences in fish species, processing methods, and origin may significantly affect the content of various nutrients and the behavioral expression of its protein extracts. There are also unstudied dried fish species and therefore relevant research gaps remain Also, the current level of research is far from comprehensive because some indigenous, niche fish species with great nutritional potential have not been fully and comprehensively investigated for their nutritional contents. Moreover, the reported contaminants in DFs were not linked to the sampling location, making it impossible to map the contaminants to ensure food safety. Existing studies have only drawn attention to microplastic contamination in DFs, but the potential health risks are still unclear. Research reports on DFPIs are limited, although studies have examined the solubility, foaming, gelling, emulsifying, and water-holding properties of dried fish proteins (myofibrillar proteins and gelatin), but low solubility limits their production. There is also lack of research activities on the functional behavior of proteins at different pH values, which could enhance utilization in different food formulations.

Therefore, the current research work was designed to study the chemical composition of dried fish samples including vitamins, heavy metals, etc., that were collected from different coastal or inland cities in Bangladesh. This study can fill some gaps in this research field with respect to the effect of location and dry fish preparation method on nutritional quality. Knowledge of the chemical composition can contribute to a proper understanding of the

nutritional properties of DFs, which could be used to formulate guidelines that will alleviate the prevalence of malnutrition in Bangladesh as well as other countries around the world. Information generated from this study could enable evaluation of the health hazards related to DF consumption, and contribute to building a database of the nutrient value of protein-rich foods. In addition, this study's analysis of the functional properties of DFPIs suggests possible new value-added products that could generate economic benefits for the DF industry.

REFERENCES

Ahmed, I., Jan, K., Fatma, S., & Dawood, M. A. O. (2022). Muscle proximate composition of various food fish species and their nutritional significance: A review. *Journal of Animal Physiology and Animal Nutrition*, *106*(3), 690–719. https://doi.org/10.1111/jpn.13711

Akter, S., Fardous, Z., Aktar, J., Rahman, M. A., Chowdhury, M. A. Z., Sarkar, S., & Billah, M. B. (2019). Environmental contamination of heavy metals in some dried fish of coastal regions of Bangladesh. *Bangladesh Journal of Zoology*, *47*(2), 293–304. https://doi.org/10.3329/bjz.v47i2.44340

Al Banna, Md. H., Hoque, Md. S., Tamanna, F., Hasan, Md. M., Mondal, P., Hossain, Md. B., Chakma, S., Jaman, Mst. N., Tareq, Md. A., & Khan, M. S. I. (2022). Nutritional, microbial and various quality aspects of common dried fish from commercial fish drying centers in Bangladesh. *Heliyon*, *8*(10), e10830. https://doi.org/10.1016/j.heliyon.2022.e10830

Alahmad, K., Xia, W., Jiang, Q., & Xu, Y. (2021). Influence of Drying Techniques on the Physicochemical, Nutritional, and Morphological Properties of Bighead Carp (*Hypophthalmichthys nobilis*) Fillets. *Foods*, *10*(11), 2837. https://doi.org/10.3390/foods10112837

Albaris B. Tahiluddin, & Ali Eslem Kadak. (2022). Traditional Fish Processing Techniques Applied in the Philippines and Turkey. *Menba Journal of Fisheries Faculty*, *8*(1), 50–58.

Amascual, R. H., Panganoron, H. O., Pajarillo, N. D., & Irene, E. A. (2020). Histamine profile of dried-salted fish sold in local supermarkets of Samar, Philippines. *Ital J Food Saf.*, *9*(1).

Azam, K., Basher, M. Z., Asaduzzaman, M., Hossain, M. H., & Ali, M. Y. (2003). Biochemical quality assessment of fourteen selected dried fish. *Univ. J. Zool.*, *22*, 23–26. https://www.researchgate.net/publication/233885730_Biochemical_assessment_of_fourteen_selected_dried_fish?enrichId=rgreq-37c7ef5e2f6c69ff2543cff78a18a2a8-

XXX&enrichSource=Y292ZXJQYWdlOzIzMzg4NTczMDtBUzoxMDExMjA1Nzk5OTc3MTFAMTQwMTEyMDQzMzM3NQ%3D%3D&el=1_x_2&_esc=publicationCoverPdf

Belton, B., Johnson, D. S., Thrift, E., Olsen, J., Hossain, M. A. R., & Thilsted, S. H. (2022). Dried fish at the intersection of food science, economy, and culture: A global survey. *Fish and Fisheries*, *23*(4), 941–962. https://doi.org/10.1111/faf.12664

Bhowmik, S., Zakaria, M. A., Sarwar, Md. S., Shofi, S. B., Syduzzaman, Akter, F., Islam, Md. M., & Mamun, A.-A. (2022). Development and nutritional index of ready to use fish products (RUFPs) from small fish species: Future superfoods for consumers. *Applied Food Research*, *2*(1), 100111. https://doi.org/10.1016/j.afres.2022.100111

Chasapis, C. T., Ntoupa, P.-S. A., Spiliopoulou, C. A., & Stefanidou, M. E. (2020). Recent aspects of the effects of zinc on human health. *Archives of Toxicology*, *94*(5), 1443–1460. https://doi.org/10.1007/s00204-020-02702-9

Chen, P., Qiu, Y., Chen, S., Zhao, Y., Wu, Y., & Wang, Y. (2022). Insights into the effects of different drying methods on protein oxidation and degradation characteristics of golden pompano (Trachinotus ovatus). *Frontiers in Nutrition*, *9*. https://doi.org/10.3389/fnut.2022.1063836

Chukwu, O. (2009). Influences of drying methods on nutritional properties of tilapia fish (Oreochromis nilotieus). *World Journal of Agricultural Sciences*, *5*(2), 256–258.

Cormick, G., & Belizán, J. M. (2019). Calcium Intake and Health. *Nutrients*, *11*(7), 1606. https://doi.org/10.3390/nu11071606

Coskuntuna, L., Gecgel, U., Yilmaz, I., Gecgel, U., & Dulger, G. C. (2015). Erratum to: Investigating Fatty Acid Composition of Various Meat and Offal Products from Turkey. *Journal of the American Oil Chemists' Society*, *92*(5), 667–667. https://doi.org/10.1007/s11746-015-2637-0

Dharmadasa, R. M., Lintha, A., Wijesekara, R. G. S., Abeysinhe, D. C., & Ranasinghe, P. (2019). Use of Halosarcia indica (Willd.) Paul G. Wilson Extracts for Low Salted Dried Fish Production. *World*, *7*(4), 132–136.

EFSA. (2010). Scientific opinion on dietary reference values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol. *EFSA Journal*, *8*(3). https://doi.org/10.2903/j.efsa.2010.1461

Emmanuel, A. T., David, O. O., & Oyeniyi, A. T. (2020). Effects of Processing Temperature on the Proximate and Mineral Composition of Oreochromis niloticus. . . *International Journal of Recent Innovation in Food Science & Nutrition*, *3*(1).

Fairweather-Tait, S., & Sharp, P. (2021). *Iron* (pp. 219–250). https://doi.org/10.1016/bs.afnr.2021.01.002

FAO. (2018). *Impacts of climate change on fisheries and aquaculture*.

FAO Expert Consultation. (2011). *Dietary protein quality evaluation in human nutrition*. https://www.fao.org/ag/humannutrition/35978-02317b979a686a57aa4593304ffc17f06.pdf

FAO, & WHO. (2023). *Pesticide Index*. CODEX ALIMENTARIUS. https://www.fao.org/fao-who-codexalimentarius/codex-texts/dbs/pestres/pesticides/en/

Fiedler, J. L., Lividini, K., Drummond, E., & Thilsted, S. H. (2016). Strengthening the contribution of aquaculture to food and nutrition security: The potential of a vitamin A-rich, small fish in Bangladesh. *Aquaculture*, *452*, 291–303. https://doi.org/10.1016/j.aquaculture.2015.11.004

Foline, O. F., Rachael, A. M., Iyabo, B. E., & Fidelis, A. E. (2011). Proximate composition of catfish (Clarias gariepinus) smoked in Nigerian stored products research institute (NSPRI): Developed kiln. *International Journal of Fisheries and Aquaculture*, *3*(5), 96–98.

Food and Nutrition Board, & Institute of Medicine. (2001a). Copper. In *Dietary reference intakes for vitamin*

A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. National Academy of Medicine.

Food and Nutrition Board, & Institute of Medicine. (2001b). Iron. In *Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc* (pp. 290–393). National Academy of Medicine.

Food and Nutrition Board, & Institute of Medicine. (2001c). Zinc. In *Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc* (pp. 442–501). National Academy of Medicine.

Gehring, C. K., Gigliotti, J. C., Moritz, J. S., Tou, J. C., & Jaczynski, J. (2011). Functional and nutritional characteristics of proteins and lipids recovered by isoelectric processing of fish by-products and low-value fish: A review. *Food Chemistry*, *124*(2), 422–431. https://doi.org/10.1016/j.foodchem.2010.06.078

Ghosh, P., Ahmed, Z., Alam, R., Begum, B. A., Akter, S., & Jolly, Y. N. (2021). Bioaccumulation of metals in selected cultured fish species and human health risk assessment: a study in Mymensingh Sadar Upazila, Bangladesh. *Stochastic Environmental Research and Risk Assessment*, *35*(11), 2287–2301. https://doi.org/10.1007/s00477-021-02026-9

Giménez, B., Gómez-Guillén, M. C., & Montero, P. (2005). Storage of dried fish skins on quality characteristics of extracted gelatin. *Food Hydrocolloids*, *19*(6), 958–963. https://doi.org/10.1016/j.foodhyd.2004.12.012

Gupta, S., Brazier, A. K. M., & Lowe, N. M. (2020). Zinc deficiency in low- and middle-income countries: prevalence and approaches for mitigation. *Journal of Human Nutrition and Dietetics*, *33*(5), 624–643. https://doi.org/10.1111/jhn.12791

Haque, E., Kamruzzaman, M., Islam, M. S., Sarwar, T., Rahman, S. S., & Karim, M. R. (2013). Assessment and comparison of quality of solar tunnel dried Bombay duck and Silver pomfret with traditional sun dried samples. *International Journal of Nutrition and Food Sciences*, *2*(4), 187–195.

Hasan, J., Dristy, E. Y., Anjumanara, Mondal, P., Hoque, M. S., Sumon, K. A., Hossain, M. A. R., & Shahjahan, M. (2023). Dried fish more prone to microplastics contamination over fresh fish – Higher potential of trophic transfer to human body. *Ecotoxicology and Environmental Safety*, *250*, 114510. https://doi.org/10.1016/j.ecoenv.2023.114510

Hasan, J., Lima, R. A., & Shaha, D. C. (2021). Fisheries resources of Bangladesh: A review. *International Journal of Fisheries and Aquatic Studies*, *9*(4), 131–138. https://doi.org/10.22271/fish.2021.v9.i4b.2532

Hashimoto, T., Suzuki, T., Hagiwara, T., & Takai, R. (2004). Study on the glass transition for several processed fish muscles and its protein fractions using differential scanning calorimetry. *Fisheries Science*, *70*(6), 1144–1152. https://doi.org/10.1111/j.1444-2906.2004.00916.x

Hoque, Md. S., Tamanna, F., Hasan, Md. M., Al Banna, Md. H., Mondal, P., Prodhan, M. D. H., Rahman, Md. Z., & van Brakel, M. L. (2022). Probabilistic public health risks associated with pesticides and heavy metal exposure through consumption of common dried fish in coastal regions of Bangladesh. *Environmental Science and Pollution Research*, *29*(14), 20112–20127. https://doi.org/10.1007/s11356-021-17127-9

Hossain, M. N., Jamil, M. G. M., Mia, M. M., Uddin, M. N., & Mansur, M. A. (2017). Studies on the proximate composition, quality and heavy metal concentration of two sun-dried marine fish (sun-dried Silver Pomfret and sun-dried Perch) of Cox's Bazar District of Bangladesh. *Journal of Environmental Science and Natural Resources*, *10*(1), 25–32.

Hossain, S., Chowdhury, P. B., Biswas, R. K., & Hossain, Md. A. (2020). Malnutrition status of children under 5 years in Bangladesh: A sociodemographic assessment. *Children and Youth Services Review*, *117*, 105291. https://doi.org/10.1016/j.childyouth.2020.105291

Iko Afé, O. H., Saegerman, C., Kpoclou, Y. E., Douny, C., Igout, A., Mahillon, J., Anihouvi, V. B., Hounhouigan, D. J., & Scippo, M.-L. (2021). Contamination of smoked fish and smoked-dried fish with polycyclic aromatic hydrocarbons and biogenic amines and risk assessment for the Beninese consumers. *Food Control*, *126*, 108089. https://doi.org/10.1016/j.foodcont.2021.108089

Immaculate, J., Sinduja, P., & Jamila, P. (2012). Biochemical and microbial qualities of Sardinella fimbriata sun dried in different methods. *International Food Research Journal*, *19*(4).

Institute of Medicine (US) Standing Committee. (1997). Calcium. In National Academies Press (US) (Ed.), *Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride*. National Academies Press (US).

Irshad, S., Muhammad Shahbaz, H., Nawaz, A., Rizwan Khan, M., Widyanti Harlina, P., Muhammad Aadil, R., Walayat, N., & Siddiqui, S. A. (2023). Effect of hydrophilic and hydrophobic emulsifiers on physio-chemical, structural and water distribution properties of meat-based cookies. *Journal of King Saud University - Science*, *35*(7), 102852. https://doi.org/10.1016/j.jksus.2023.102852

Islam, S., Bhowmik, S., Majumdar, P. R., Srzednicki, G., Rahman, M., & Hossain, Md. A. (2021). Nutritional profile of wild, pond-, gher- and cage-cultured tilapia in Bangladesh. *Heliyon*, *7*(5), e06968. https://doi.org/10.1016/j.heliyon.2021.e06968

Jiao, L., Tu, C., Mao, J., Benjakul, S., & Zhang, B. (2022). Impact of theaflavin soaking pretreatment on oxidative stabilities and physicochemical properties of semi-dried large yellow croaker (Pseudosciaena crocea) fillets during storage. *Food Packaging and Shelf Life*, *32*, 100852.

https://doi.org/10.1016/j.fpsl.2022.100852

Junianto, ., Brainerd, E., Maghfira, R., Suyono, M. L. A., Rizki, A. F., Pratama, R. L., & Barkah, S. M. (2022). Utilization of Fish Bone Waste for Food. *Asian Journal of Fisheries and Aquatic Research*, 46–56. https://doi.org/10.9734/ajfar/2022/v20i2493

Kar, M., Hoq, Md. E., Islam, Md. S., Islam, Md. M., Meghla, N. T., Suravi, S., & Kabir, Md. H. (2020). Monitoring of Proximate Composition, Heavy Metal Concentrations and Pesticide Residues in Marine Dried Fish Available in the Coastal Region of Bangladesh. *Grassroots Journal of Natural Resources*, *3*(2), 30–41. https://doi.org/10.33002/nr2581.6853.03023

Karami, A., Golieskardi, A., Ho, Y. Bin, Larat, V., & Salamatinia, B. (2017). Microplastics in eviscerated flesh and excised organs of dried fish. *Scientific Reports*, *7*(1), 5473. https://doi.org/10.1038/s41598-017-05828-6

Khalili Tilami, S., & Sampels, S. (2018). Nutritional Value of Fish: Lipids, Proteins, Vitamins, and Minerals. *Reviews in Fisheries Science & Aquaculture*, *26*(2), 243–253. https://doi.org/10.1080/23308249.2017.1399104

Kubra, K., Azam, M., Hoque, Md. S., Sharker, Md. R., & Hossen, S. (2020). Fish Drying and Socio-Economic Condition of Dried Fish Producers in the Coastal Region of Bangladesh. *Middle-East Journal of Scientific Research*, *28*(3), 182–192.

Kutralam-Muniasamy, G., Shruti, V. C., Pérez-Guevara, F., Roy, P. D., & Martínez, I. E. (2023). Consumption of commercially sold dried fish snack “Charales” contaminated with microplastics in Mexico. *Environmental Pollution*, *332*, 121961. https://doi.org/10.1016/j.envpol.2023.121961

Lee, W., Clark, S., & Swanson, B. G. (2006). FUNCTIONAL PROPERTIES OF HIGH HYDROSTATIC PRESSURE-TREATED WHEY PROTEIN. *Journal of Food Processing and Preservation*, *30*(4), 488–

501. https://doi.org/10.1111/j.1745-4549.2005.00081.x

Lewis, N. A., Daniels, D., Calder, P. C., Castell, L. M., & Pedlar, C. R. (2020). Are There Benefits from the Use of Fish Oil Supplements in Athletes? A Systematic Review. *Advances in Nutrition*, *11*(5), 1300–1314. https://doi.org/10.1093/advances/nmaa050

Liu, J., Chen, J., Wang, S., Xie, J., Wang, Y., Chai, T.-T., Ong, M. K., Wu, J., Tian, L., & Bai, W. (2022). Effects of Monascus application on in vitro digestion and fermentation characteristics of fish protein. *Food Chemistry*, *377*, 132000. https://doi.org/10.1016/j.foodchem.2021.132000

Lopez, M. J., & Mohiuddin, S. S. (2023). *Biochemistry, Essential Amino Acids*. StatPearls Publishing. https://pubmed.ncbi.nlm.nih.gov/32496725/

Lou, X., Wang, C., Xiong, Y. L., Wang, B., Liu, G., & Mims, S. D. (2000). Physicochemical Stability of Paddlefish (*Polyodon spathula*) Meat Under Refrigerated and Frozen Storage. *Journal of Aquatic Food Product Technology*, *9*(4), 27–39. https://doi.org/10.1300/J030v09n04_04

Lou, X., Wang, C., Xiong, Y. L., Wang, B., & Mims, S. D. (2000). Gelation Characteristics of Paddlefish *(Polyodon spathula)* Surimi Under Different Heating Conditions. *Journal of Food Science*, *65*(3), 394–398. https://doi.org/10.1111/j.1365-2621.2000.tb16014.x

Maïworé, J., Ngoune, L. T., Koumba, M. K., Metayer, I., Montet, D., & Durand, N. (2021). Determination of bacterial population and the presence of pesticide residues from some Cameroonian smoked and dried fish. *Scientific African*, *13*, e00886. https://doi.org/10.1016/j.sciaf.2021.e00886

Mansur, M. A., Rahman, S., Khan, M. N. A., Reza, M. S., & Uga, S. (2013). Study on the quality and safety aspect of three sun-dried fish. *African Journal of Agricultural Research*, *8*(41), 5149–5155. https://doi.org/DOI: 10.5897/AJAR12.773

Mazorra-Manzano, M. A., Ramirez-Suarez, J. C., Moreno-Hernandez, J. M., & Pacheco-Aguilar, R. (2017).

Seafood proteins. In Y. R. Yada (Ed.), *Proteins in food processing* (2nd ed., pp. 445–467). Andre Gerhard Wolff.

Myint, Z. W., Oo, T. H., Thein, K. Z., Tun, A. M., & Saeed, H. (2018). Copper deficiency anemia: review article. *Annals of Hematology*, *97*(9), 1527–1534. https://doi.org/10.1007/s00277-018-3407-5

Nie, Y., Chen, J., Xu, J., Zhang, Y., Yang, M., Yang, L., Wang, X., & Zhong, J. (2022). Vacuum freeze-drying of tilapia skin affects the properties of skin and extracted gelatins. *Food Chemistry*, *374*, 131784. https://doi.org/10.1016/j.foodchem.2021.131784

Niu, J., Zhao, B., Guo, X., & Yin, T. (2019). Effects of Vacuum Freeze-Drying and Vacuum Spray-Drying on Biochemical Properties and Functionalities of Myofibrillar Proteins from Silver Carp. *Journal of Food Quality*, *2019*, 1–8. https://doi.org/10.1155/2019/9457835

Njoroge, J. G., & Lokuruka, M. N. I. (2020). SENSORY ACCEPTABILITY OF COOKIES FORTIFIED WITH TILAPIA FISH BONE POWDER. *Journal of Food and Nutritional Sciences Research*. https://doi.org/10.37512/800

Odoli, C. O., Oduor-Odote, P., & Arason, S. (2019). The influence of lipid content and pretreatment methods on protein conformation in fish (capelin, *Mallotus villosus*) during smoking and drying. *Food Science & Nutrition*, *7*(4), 1446–1454. https://doi.org/10.1002/fsn3.980

Ofuani, A. G. , & D. E. C. (2022)., & Destiny, E. C. (2022). A Comparative Analysis on the Use of DDT, Dichlorvos, Endosulfan, Heptachlor and Chlorpyrifos in the Preservation of Smoked-Dried Fish in Ogwashi-Uku, Delta State. *IPS Journal of Public Health*, *1*(2), 13–16.

Ogbonnaya, C., & Shaba, M. I. (2009). Effects of drying methods on proximate compositions of catfish (Clarias gariepinus). *World Journal of Agricultural Sciences* , *5*(1), 114–116.

Ogunbambo, M. M. (2020). Fatty acid processing yield of smoke-dried clarias gariepinus (Burchell, 1822)

using two different smoking kilns at varying temperatures. *Animal Research International*, *17*(2), 3729–3735.

Olokor, J. O., & Ngwu, E. O. (2009). *Study of the solar dried and traditional sun-dried fish products*.

Paul, P. C., Reza, M. S., Islam, M. N., & Kamal, M. (2018). A review on dried fish processing and marketing in the coastal region of Bangladesh. *Research in Agriculture Livestock and Fisheries*, *5*(3), 381–390. https://doi.org/10.3329/ralf.v5i3.39587

Penniston, K. L., & Tanumihardjo, S. A. (2003). Vitamin A in dietary supplements and fortified foods: Too much of a good thing? *Journal of the American Dietetic Association*, *103*(9), 1185–1187. https://doi.org/10.1016/S0002-8223(03)00978-7

Phillips, C. M., Kesse-Guyot, E., McManus, R., Hercberg, S., Lairon, D., Planells, R., & Roche, H. M. (2012). High Dietary Saturated Fat Intake Accentuates Obesity Risk Associated with the Fat Mass and Obesity-Associated Gene in Adults. *The Journal of Nutrition*, *142*(5), 824–831. https://doi.org/10.3945/jn.111.153460

Pradhan, N., Shrestha, M. K., Rai, S., Jha, D. K., & Sah, S. K. (2018). Quality assessment of dried mrigal (Cirrhunus mrigala) chhari fish from different market outlets of Nepal. *International Journal of Fisheries and Aquatic Studies*, *6*(2), 463–471.

Raghunath, M. R., Sankar, T. V, Ammu, K., & Devadasan, K. (1995). Biochemical and nutritional changes in fish proteins during drying. *Journal of the Science of Food and Agriculture*, *67*(2), 197–204. https://doi.org/10.1002/jsfa.2740670209

Rahman, M. A., Saifullah, M., & Islam, M. N. (2012). Fish powder in instant fish soup mix. *Journal of the Bangladesh Agricultural University*, *10*(452-2016–35550), 145–148.

Rakib, Md. R. J., Jolly, Y. N., Enyoh, C. E., Khandaker, M. U., Hossain, M. B., Akther, S., Alsubaie, A.,

Almalki, A. S. A., & Bradley, D. A. (2021). Levels and health risk assessment of heavy metals in dried fish consumed in Bangladesh. *Scientific Reports*, *11*(1), 14642. https://doi.org/10.1038/s41598-021-93989-w

Rana, M. M., Chakraborty, S. C., & Saeid, A. (2020). Comparative Studies of Nutritional, Microbial and Organoleptic Properties of Different Indigenous Dried Fish from Local Market in Bangladesh. *Advanced Journal of Chemistry-Section A*, *3*(3), 318–327. https://doi.org/10.33945/SAMI/AJCA.2020.3.9

Rice, A. L., West Jr, K. P., & Black, R. E. (2004). Vitamin A deficiency. In *Comparative quantification of health risks: global and regional burden of disease attributable to selected major risk factors* (pp. 0211–0256).

Rukmangada, R., Naidu, B. C., Nayak, B. B., Balange, A., Chouksey, M. K., & Xavier, K. A. M. (2023). Microplastic contamination in salted and sun dried fish and implications for food security – A study on the effect of location, style and constituents of dried fish on microplastics load. *Marine Pollution Bulletin*, *191*, 114909. https://doi.org/10.1016/j.marpolbul.2023.114909

Sablani, S. S., Myhara, R. M., Mahgoub, Al-Attabi, Z. H., & Al-Mugheiry, M. M. (2001). Water sorption isotherms of freeze dried fish sardines. *Drying Technology*, *19*(3–4), 673–680. https://doi.org/10.1081/DRT-100103943

Sarkardei, S., & Howell, N. K. (2007). The effects of freeze-drying and storage on the FT-Raman spectra of Atlantic mackerel (Scomber scombrus) and horse mackerel (Trachurus trachurus). *Food Chemistry*, *103*(1), 62–70. https://doi.org/10.1016/j.foodchem.2006.06.065

Scott, K. C., & Latshaw, J. D. (1994). The vitamin D3 and precholecalciferol content of menhaden fish meal as affected by drying conditions. *Animal Feed Science and Technology*, *47*(1–2), 99–105.

https://doi.org/10.1016/0377-8401(94)90163-5

Shamsuzzaman, Md. M., Islam, M. M., Tania, N. J., Abdullah Al-Mamun, Md., Barman, P. P., & Xu, X. (2017). Fisheries resources of Bangladesh: Present status and future direction. *Aquaculture and Fisheries*, *2*(4), 145–156. https://doi.org/10.1016/j.aaf.2017.03.006

Shaviklo, G. R., Thorkelsson, G., Arason, S., & Sveinsdottir, K. (2012). Characteristics of freeze-dried fish protein isolated from saithe (Pollachius virens). *Journal of Food Science and Technology*, *49*(3), 309–318. https://doi.org/10.1007/s13197-011-0285-4

Siddhnath, Ranjan, A., Mohanty, B. P., Saklani, P., Dora, K. C., & Chowdhury, S. (2022). Dry Fish and Its Contribution Towards Food and Nutritional Security. *Food Reviews International*, *38*(4), 508–536. https://doi.org/10.1080/87559129.2020.1737708

Slavin, M., Dong, M., & Gewa, C. (2016). Effect of clove extract pretreatment and drying conditions on lipid oxidation and sensory discrimination of dried omena (*Rastrineobola argentea*) fish. *International Journal of Food Science & Technology*, *51*(11), 2376–2385. https://doi.org/10.1111/ijfs.13218

Sroy, S., Avallone, S., Servent, A., In, S., & Arnaud, E. (2023). Does drying preserve the nutritional quality of small freshwater fish without excessive concentrations of heavy metals? *Current Research in Food Science*, *6*, 100489. https://doi.org/10.1016/j.crfs.2023.100489

Tenyang, N., Ponka, R., Tiencheu, B., Djikeng, F. T., & Womeni, H. M. (2020). Effect of Traditional Drying Methods on Proximate Composition, Fatty Acid Profile, and Oil Oxidation of Fish Species Consumed in the Far-North of Cameroon. *Global Challenges*, *4*(8), 2000007. https://doi.org/10.1002/gch2.202000007

Uddin, K. B. , Reza, M. S., Shikha, F. H., & Kamal, M. (2017). Influence of salt concentration on nutritional quality of solar tunnel dried silver jewfish (Pennahia argentata). *International Journal of Fisheries and*

Aquatic Studies, *53*, 571–575.

Ullah, S., Zuberi, A., Alagawany, M., Farag, M. R., Dadar, M., Karthik, K., Tiwari, R., Dhama, K., & Iqbal, H. M. N. (2018). Cypermethrin induced toxicities in fish and adverse health outcomes: Its prevention and control measure adaptation. *Journal of Environmental Management*, *206*, 863–871. https://doi.org/10.1016/j.jenvman.2017.11.076

Wood, J. D., Enser, M., Fisher, A. V., Nute, G. R., Sheard, P. R., Richardson, R. I., Hughes, S. I., & Whittington, F. M. (2008). Fat deposition, fatty acid composition and meat quality: A review. *Meat Science*, *78*(4), 343–358. https://doi.org/10.1016/j.meatsci.2007.07.019

CHAPTER THREE

3 NUTRITIONAL QUALITY OF DRIED FISH FROM BANGLADESH

Huan Sun [1], Derek S. Johnson [2], Rotimi E. Aluko [1, 3, *]

*****Corresponding author. E-mail address: rotimi.aluko@umanitoba.ca (Rotimi E. Aluko)

1 Department of Food and Human Nutritional Sciences, University of Manitoba, Room 209 Human Ecology Building, 35 Chancellor's Circle, Winnipeg, MB R3T 2N2, Canada

2 Department of Anthropology, University of Manitoba, 432 Fletcher Argue Building, 15 Chancellor Circle, Winnipeg, MB R3T 2N2, Canada

3 Richardson Centre for Food Technology and Research, University of Manitoba, 196 Innovation Dr, Winnipeg, MB R3T 2N2, Canada

Contribution of Authors statements

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

3.1 Introduction

Global fishery and aquatic production reached a record high of 122.6 million tons in 2020 (*The State of World Fisheries and Aquaculture 2022*, 2022). With the current and foreseeable increase in global per capita consumption of aquatic foods, the Food and Agriculture Organization of the United Nations (FAO) reported that the fishery sector continually plays a crucial role in meeting the needs of population growth-related food stress and nutritional requirements of the future (*The State of World Fisheries and Aquaculture 2022*, 2022). Asia is the center of global fishery production, accounting for 70% of the world's total production, and is where some of the top-ten fish production countries in the world namely, China, Indonesia, India, Vietnam, Japan, and Bangladesh are located (*The State of World Fisheries and Aquaculture 2022*, 2022). Among them, Bangladesh's fishery industry is unique, as its production capacity is highly dependent on inland aquaculture, accounting for 56.76% of the country's total production (Hasan et al., 2021). In facing the world food crisis, the FAO has highlighted the importance of sustainability in fisheries to alleviate hunger and nourish people worldwide (*The State of World Fisheries and Aquaculture 2022*, 2022). Therefore, research on the quality of Bangladeshi fishery products will contribute to fishery sustainability and food security.

Malnutrition is widespread worldwide, leaving newborns, children, and adolescents immune deficient and more susceptible to diseases (Black et al., 2013). In Bangladesh, 36% of children under 5 years of age are stunted and the wasting rate of 14% is among the highest in the world, which is also rooted in malnutrition (NIPORT & Macro, 2005; WHO & UNICEF, 2017). Deficiencies in micronutrients such as 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 further exacerbated by poor dietary diversity (FAO, 2013; Nordhagen et al., 2020). The poor population of Bangladesh faces serious malnutrition problems due to lack of high-quality dietary proteins from animal and plant sources (Rahman et al., 2012). And among them, iron deficiency anemia in pregnant women and children is a special public health challenge that is prevalent in Bangladesh (ICDDRB, 2013).

Sun-dried fishes are considered nutrient-rich sources of proteins, lipids, minerals, and vitamins (Bhowmik et al., 2022). In addition, as one of the main forms of stored fish, dried fishes (DFs) have a long history of production and consumption in Bangladesh. It has been reported that Bengal DFs are rich in protein (more than 50% dry weight), calcium, iron, zinc and n-3 LC-PUFA with health benefits, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Bhowmik et al., 2022). And because the price per unit of protein in DFs is cheaper than that of fresh fish (Hossain et al., 2017), consumption of dried fish is considered to alleviate the public health pressure caused by the prevalence of malnutrition among vulnerable groups.

However, even though there are multiple studies that have contributed to understanding the nutrient aspect of the DFs, gaps still exist in the field as most of the studies focused on sampling in specific areas and the reported data relate mainly to the proximate composition. More in-depth and comprehensive systematic nutritional research is lacking. Therefore, this study focused on analyzing seven DFs collected from four cities in Bangladesh to conduct a systematic nutritional evaluation including proximate, mineral, heavy metals, amino acids, fatty acids, vitamin B_{12}, and cholesterol composition and estimate the potential contribution of

100 g DFs/day consumption to the estimated average requirement (EAR) of the population most in need.

3.2 Materials and methods

3.2.1 Raw materials and sample preparation

Seven different types of dried fish were purchased from four local markets and then transported to the laboratory. Upon arrival, the samples were stored at -20°C until used for the experiments. Information on the dried fish samples is given in the Table 1. 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 fishes were thawed at 4 ℃ overnight, then oven dried (Isotemp oven 516, Fisher Scientific, CA) at 50℃ overnight. The DFs were then ground into a fine powder using a coffee grinder, and stored in airtight containers at -20°C.

Table 1. Sample names and sampling location information for seven Bangladeshi dried fish

Sample name	Species name	Sampling location	Sample ID
Bombay duck	*Harpadon nehereus*	Cox's Bazar	BD-C
		Dhaka	BD-D
		Mymensingh	BD-M
Ribbon fish	*Trichiurus lepturus*	Cox's Bazar	RF-C
		Dhaka	RF-D
		Mymensingh	RF-M
White sardine	*Escualosa thoracata*	Cox's Bazar	WS-C
		Dhaka	WS-D
		Mymensingh	WS-M
Freshwater barb	*Puntius spp.*	Sylhet	FB-S
		Dhaka	FB-D
		Mymensingh	FB-M
Ganges River sprat	*Corica soborna*	Sylhet	GR-S
		Dhaka	GR-D
		Mymensingh	GR-M
Fermented barb	*Puntius spp.*	Sylhet	FM-S
		Dhaka	FM-D
		Mymensingh	FM-M
Fermented anchovy	*Setipnna spp.*	Sylhet	FA-S
		Dhaka	FA-D
		Mymensingh	FA-M

3.2.2 Proximate and mineral composition analysis

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

3.2.3 Heavy metals

Mercury (Hg) content was determined by US EPA method 7473, conducted on a Hydra IIC (TELEDYNE, Leeman Labs, Combustion). 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).

3.2.4 Amino acid composition

The amino acid profile of the DFs was 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 performing 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 (>18 years old, FAO Expert Consultation, 2011). That is, 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:

DEAAS (%) = min [percentage contribution of histidine, leucine, isoleucine, lysine, threonine, tryptophan, valine, sulfur containing amino acid, aromatic amino acids] (FAO Expert Consultation, 2011).

3.2.5 Muscle protein composition

The extraction of sarcoplasmic and myofibrillar proteins was performed using the method described by Hashimoto et al. (1979). The protein content of each fraction was determined by the modified Lowry method (Markwell et al., 1978).

3.2.6 Free amino acids

The free amino acids extraction followed the method of egg yolk free amino acids extraction (Goto et al., 2021). Identification of free amino acids were carried out using the Pico-Tag method (Bidlingmeyer et al. 1984).

3.2.7 In vitro protein digestibility

The *in vitro* digestibility was determined using the 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 M NaOH while stirring at 37 ºC. One milliliter of an enzyme mixture (4.8 mg/ml trypsin, 9.3 mg/ml chymotrypsin, 3.9 mg/ml peptidase)) was then added to the 30 ml 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 percent protein digestibility of each DF was determined as follows using the regression equation of Hsu et al. (1977).

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

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

3.2.8 Fatty acids composition

The fish lipids were extracted following a standard protocol (Foloch et al., 1957). Briefly, 1 g of DF was mixed with 4 ml of 0.025 $CaCl_2$ 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 BF_3 methanol solution (14%, w/w) 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 double distilled water (DDW) 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 was collected into a pre-weighed 12 ml glass tube (W_1), then evaporated with nitrogen at 40 °C water bath. The weight of the tube with lipids was recorded as W_2 and the total weight of the lipid was calculated as

$$Total\ lipd\ weight = W2 - W1$$

A 10 mg/ml of lipid solution was prepared by diluting the methylated lipids in hexane and then injected into a gas chromatography system (450-GC; Bruker, Canada). The GC included a flame ionization detector and a DB225MS column (30 m x 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.

3.2.9 Cholesterol content

The cholesterol was determined by the protocol outlined by Van Elswyk et al. (1991) and Rudel & Morris (1973) with some modifications. Briefly, 200 mg of DF powder was mixed with 1.6 ml of 50% (w/v) KOH and 8 ml of 95% ethanol in a capped glass centrifuge tube and incubated in a 40 ℃ water bath for 1 h. The mixture was cooled to room temperature, 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 extracted with 12 ml of hexane for a total of three times. The hexane layers were combined and washed five times by adding 10 ml of DDW. 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 ℃ water bath. One milliliter of cholesterol

standard (2 mg/ml ethanol) was prepared the same way with the DF and 0, 45, 90, 180, 900, 1800 and 3600 µl aliquot of standard was evaporated with nitrogen flush in the 40 ℃ water bath. Two milliliters 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 milliliter of concentrated sulfuric acid was added into the mixture, then thoroughly mixed and incubated in the dark for 10 min. Absorbances vs. concentrations of the standard at 550 nm were used to obtain a linear regression equation. The cholesterol content of DF samples was calculated using the regression equation.

3.2.10 Vitamin B_{12} content

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

3.2.11 Statistical analysis

In this study, all presented data were collected from duplicate analyses. Data was analyzed through one-way and two-way ANOVA using IBM SPSS 28.0.0.0 (IBM, 2023) and reported as mean ± standard deviation. The statistical differences were determined by Duncan's multiple range test (p<0.05).

3.3 Results and discussion

3.3.1 Proximate composition

The proximate composition (moisture, protein, fat, and ash) of the DFs is as shown in Table 2.

3.3.1.1 Moisture

The moisture content of DFs in this study ranged between 4.6-16.7%, which is consistent with the results reported by Bhowmik et al. (2022). According to Haider et al. (2021) and van

Ruth et al. (2014), less than 20% moisture content is beneficial for limiting the growth of microbes. This moisture range helps to maintain the quality of the DFs, which is considered a required condition for long-term preservation and eating quality. Therefore, DFs in this study are considered to have the prerequisites for long-term shelf life without the possibility of adverse microbial contamination. In addition, by comparing among the sampling locations (SLs; data not shown), the results showed that DFs from Cox's Bazar have the highest moisture content, which is consistent with the findings of Kar et al. (2020).

3.3.1.2 Protein

The protein content of the DFs varied by DF types and SLs, with values that ranged between 44.3 and 75.7%, which is similar to the 51-77% of a previous report (Kar et al., 2020). The higher protein contents of WS and GR fish samples makes them excellent protein-rich food materials. Fish protein is considered a complete protein as it contains all the essential amino acids required for the normal functioning of major organs of the body such as brain, hearts, and eyes (Hei, 2021). In addition, consumption of fish protein has many other health benefits, according to Vikøren et al. (2013) who reported that a short-term daily supplementation with low doses of fish protein may have beneficial effects on blood glucose and LDL cholesterol levels as well as glucose tolerance and body composition in overweight adults. Another study reported that β-parvalbumin in fish protein may have the potential to inhibit the formation of amyloid-related neurodegenerative diseases such as in Alzheimer's and Parkinson's by inhibiting the formation of amyloid structures (Werner et al., 2018).

3.3.1.3 Fat

The fat content of the DFs was within the range of 2.9-20.6%, and was significantly

affected by fish species. The FB and FM with the highest fat content 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%, 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 compared with Kachiki (*Corica soborna*), Churi (*Trichiurus lepturus*), and Loita (*Harpadon nehereus*). However, it should be noted that the fat content will be affected by various factors apart from species, such as diet, temperature, salinity, selective mobilization and distribution (Lovern, 1950).

3.3.1.4 Ash

The ash content of the DFs ranged from 11.5-31.5%. According to Bhowmik et al. (2022), the high ash content of dried fish mainly comes from viscera, bones and fins. Therefore, fish species can be a main factor that determine the ash content. In this study, FB and FM made by *Puntius spp.* and FA made by *Setipnna spp.* were found to have a higher ash content, which is similar to the results reported by previous studies (Kar et al., 2020; Rana et al., 2020). In addition, the finding that CDFs (Cox's Bazar dried fishes) contain a higher ash content is consistent with the report of Kar et al. (2020); therefore, it is possible that the ash content is also related to the sampling location. Considering that salt is added to the fish as a preservative to ensure that the meat is not spoiled during production and storage (Paul et al., 2018), the high ash content of dried fish from a certain origin may be caused by excessive amounts of added salt.

Table 2. Proximate composition of dried fishes obtained from Bangladesh

Sample ID	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
BD-C	$10.68 \pm 0.25^{b, 1}$	$58.33 \pm 0.08^{c, 2}$	$5.99 \pm 0.20^{b, 3}$	$26.50 \pm 0.29^{b, 1}$
BD-D	$10.91 \pm 0.01^{b, 1}$	$65.21 \pm 0.04^{c, 1}$	$14.98 \pm 0.30^{b, 2}$	$11.72 \pm 0.10^{b, 2}$
BD-M	$8.32 \pm 0.00^{b, 2}$	$64.32 \pm 0.66^{c, 1}$	$17.36 \pm 0.16^{b, 1}$	$11.67 \pm 0.08^{b, 2}$
RF-C	$16.68 \pm 0.54^{a, 1}$	$58.72 \pm 0.08^{b, 3}$	$5.94 \pm 0.20^{d, 1}$	$22.36 \pm 0.08^{b, 1}$
RF-D	$12.7 \pm 0.24^{a, 2}$	$69.16 \pm 2.48^{b, 2}$	$2.91 \pm 0.32^{d, 2}$	$17.25 \pm 0.80^{b, 2}$
RF-M	$7.94 \pm 0.11^{a, 3}$	$75.46 \pm 1.43^{b, 1}$	$1.94 \pm 0.08^{d, 3}$	$17.06 \pm 1.08^{b, 2}$
WS-C	$11.28 \pm 0.13^{b, 1}$	$66.26 \pm 0.88^{a, 2}$	$3.78 \pm 0.08^{d, 1}$	$21.65 \pm 0.17^{b, 1}$
WS-D	$9.11 \pm 0.04^{b, 3}$	$75.48 \pm 0.23^{a, 1}$	$2.90 \pm 0.31^{d, 2}$	$14.89 \pm 0.08^{b, 2}$
WS-M	$9.56 \pm 0.19^{b, 2}$	$75.65 \pm 0.13^{a, 1}$	$3.68 \pm 0.11^{d, 1}$	$14.24 \pm 0.17^{b, 3}$
FB-S	$8.36 \pm 0.08^{d, 1}$	$53.62 \pm 0.71^{d, 1}$	$10.86 \pm 0.23^{a, 3}$	$28.92 \pm 0.23^{a, 1}$
FB-D	$5.04 \pm 0.10^{d, 2}$	$47.00 \pm 0.05^{d, 3}$	$27.71 \pm 0.37^{a, 1}$	$21.02 \pm 0.20^{a, 3}$
FB-M	$4.60 \pm 0.11^{d, 3}$	$51.41 \pm 0.19^{d, 2}$	$19.19 \pm 0.11^{a, 2}$	$25.78 \pm 0.24^{a, 2}$
GR-S	$7.39 \pm 0.37^{d, 1}$	$72.36 \pm 0.25^{a, 2}$	$9.92 \pm 0.07^{cd, 1}$	$11.47 \pm 0.17^{c, 2}$
GR-D	$6.51 \pm 0.25^{d, 2}$	$75.15 \pm 0.21^{a, 1}$	$6.58 \pm 0.22^{cd, 3}$	$12.97 \pm 0.30^{c, 1}$
GR-M	$6.30 \pm 0.02^{d, 2}$	$75.17 \pm 1.10^{a, 1}$	$7.81 \pm 0.02^{cd, 2}$	$12.17 \pm 0.15^{c, 2}$
FM-S	$8.75 \pm 0.07^{cd, 1}$	$47.40 \pm 0.54^{e, 1}$	$19.90 \pm 0.31^{a, 1}$	$26.70 \pm 0.01^{a, 2}$
FM-D	$4.90 \pm 0.19^{cd, 2}$	$44.48 \pm 0.16^{e, 2}$	$20.57 \pm 0.07^{a, 1}$	$31.17 \pm 0.40^{a, 1}$
FM-M	$9.03 \pm 0.08^{cd, 1}$	$47.26 \pm 0.59^{e, 1}$	$19.94 \pm 1.10^{a, 1}$	$24.96 \pm 0.10^{a, 3}$
FA-S	$10.98 \pm 0.22^{bc, 1}$	$61.81 \pm 1.65^{c, 1}$	$6.60 \pm 0.19^{bc, 3}$	$23.67 \pm 0.42^{a, 2}$
FA-D	$8.88 \pm 0.08^{bc, 2}$	$54.68 \pm 0.24^{c, 1}$	$17.93 \pm 0.08^{bc, 1}$	$23.47 \pm 0.64^{a, 2}$
FA-M	$7.41 \pm 0.49^{bc, 3}$	$59.27 \pm 6.21^{c, 1}$	$9.35 \pm 0.16^{bc, 2}$	$31.49 \pm 0.05^{a, 1}$

Different letters (a, b, c, etc.) represent significant differences between fish types ($p<0.05$). Different numbers (1, 2, and 3) represent significant differences within the same fish type and different locations ($p<0.05$). BD: Bombay duck (*Harpadon nehereus*); RF: ribbon fish (*Trichiurus lepturus*); WS: white sardine (*Escualosa thoracata*); FB: freshwater barb (*Puntius spp.*); GR: Ganges River sprat (*Corica soborna*); FM: fermented barb (*Puntius spp.*); FA: fermented anchovies (*Setipnna spp.*); C: Cox's Bazar; D: Dhaka; M: Mymensingh; S: Sylhet

3.3.2 Mineral composition

The mineral composition of the DFs is summarized in Table 3, which consists of the macro-minerals (phosphorus, potassium, calcium, sodium, and magnesium) presented in weight percentage and micro-minerals (manganese, iron, copper, and zinc) reported as mg/kg.

3.3.2.1 Macro-minerals

3.3.2.1.1 Sodium

Sodium is one of the most abundant elements found in the DFs, among which FB and FM made by *Puntius spp.* had the highest level of about 7.8-4.3 g/100 g and 6.3-3.9 g/100 g, respectively. 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 powder (*Corica soborna*). As reflected in the data, the sodium content is related to the sampling locations, specifically to the local manufacturers' process. We observed that CDFs had a higher sodium content which may explain the previous finding that CDFs had a higher ash content. Studies were found to back up the finding in the present study (Kubra et al., 2020; Mandal, 2021), that during the salting procedure, which is common in sun-dried fish production, Cox’s Bazar used more salt than that in Patuakhali and Barisal region, with a salt to fish ratio of 1:12 (Cox’s Bazar) and 1:15 (Patuakhali and Barisal region). The higher sodium content of CDF also explains why it can be preserved for a long time in the presence of higher moisture content.

An appropriate amount of daily dietary sodium can maintain the normal extracellular fluid composition, and balance the water, acid-base, and salt in the body (Quintaes & Diez-Garcia, 2015). However, excessive sodium intake is known to be closely related with the onset of

hypertension, cardiovascular disease, 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 (World Health Organization, 2012). Therefore, with regard to limiting sodium intake, DFs that contain high concentration of sodium should be consumed minimally and with caution.

3.3.2.1.2 Calcium

Calcium is essential for bone and dental health, muscle contraction, nerve conduction, blood coagulation, enzyme activation, and hormone secretion (Quintaes & Diez-Garcia, 2015). In our study, the calcium contents of the DFs are comparable to that of sodium, ranging between 1.7-7.0 g/100 g. FM (4.9-6.5 g/100 g) and FA (4.8-7.0 g/100 g of DF) were found to have the most abundant amounts of calcium, which are higher than previously reported values. For example, Bhowmik et al. (2022) reported a calcium content of 3.9 g/100 g of dried Punti powder while fresh anchovies contained 3.5 g/100 g (Ullah et al., 2022). The low moisture contents of some of the DFs used in the present study may be responsible for the higher calcium levels. We observed a higher calcium level in SDFs, which may be due to the effect of pH and calcium concentration in the local water body (Wendelaar Bonga & Flik, 1993). In this study, under an assumed standard consumption portion (Bhowmik et al., 2022), 15 g/day for adults, whole DF was considered to meet at least 19.21% up to 80.82% of the calcium recommended dietary allowance (RDA, 1.3 mg/day) of adolescents (14-18 years of age) based on the United States Institute of Medicine Standing Committee (1997).

3.3.2.1.3 Potassium

Dietary potassium benefits the reduction of blood pressure, especially among people with

high-sodium diets (Weaver, 2013). The highest level of potassium was found in GR (1.9-1.7 g/100 g) while the lowest was found in FM and FA (0.7-0.6 g/100 g). The 0.6 g/100 g potassium content in Punti powder reported by Bhowmik et al. (2022), is basically consistent with the present result. However, Bhowmik et al. (2022) also reported 0.5 g/100 g potassium content for *Corica soborna* powder, which is lower than the values obtained in the current study and is probably because of the relatively low moisture content of GR. In a longitudinal follow-up study (Mosallanezhad et al., 2023), a higher sodium/potassium ratio (>0.8) was associated with an increased risk of cardiovascular diseases (CVD). In our study, only GR (0.21-0.25) and RF-D (0.63) could meet the cut-off sodium/potassium ratio. Therefore, GR and RF-D can be used as excellent potassium sources for people who have a high-sodium diet to achieve a relatively healthy sodium/potassium ratio.

3.3.2.1.4 Phosphorus and magnesium

The highest phosphorus content (2.4-3.6 g/100 g) was found in FA while the lowest (1.2-1.5 g/100 g) was in BD. Our findings are in agreement with the 2.1 g/100 g phosphorus content of fresh *Setipnna spp.* (Ullah et al., 2022) and 0.8 g/100 g of dried *Harpadon nehereus* muscle (Nazir & Magar, 1965). 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 acid (DNA and RNA) synthesis (Bird & Eskin, 2021). According to the United States Institute of Medicine Standing Committee (1997), the estimated average requirement (EAR) for phosphorus among adolescents aged 9-18 years is 1.055 g/day, which can be met at least 17.20 % up to 50.90% by consumption of 15 g of DF from this study. 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). In the present study, the magnesium level of the DFs showed no significant differences with a range of 0.33-0.15 g/100 g, which is consistent with the 0.15 and 0.18 g/100 g reported for dried Punti and dried *Corica soborna*, respectively (Bhowmik et al., 2022). According to the United States Institute of Medicine Standing Committee (1997), the EAR for the elderly (51->70 years old) is 0.42 g/day for men and 0.32 g/day for women. Fifteen grams of DFs in our study can contribute at least 5.36% up to 23.93% of the elderly magnesium EAR.

3.3.2.2 Micro-minerals

3.3.2.2.1 Zinc and manganese

Zinc is one of the predominant micro-elements in DFs, and the highest contents of 155.3-236.8 mg/kg were found in the GR. Our finding is higher than the previously reported 128.6 mg/kg for whole dried Kachki (*Corica soborna*) powder (Bhowmik et al., 2022). Zinc is crucial in mediating antioxidant and has anti-inflammatory effects on the human body (Skalny et al., 2021). Zinc deficiency was proven to relate 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% and 57% of preschool-aged children and 15-49-year-old non-lactating/pregnant women, respectively were found not to have sufficient zinc intake, which makes it one of the prevailing malnutrition conditions in Bangladesh. According to the Food and Nutrition Board & Institute of Medicine (2001), the recommended dietary allowance (RDA) of zinc for lactating 14-18-year-old women is 13 mg/day. Fifteen grams of DFs in the present study can

contribute at least 5.04% up to 27.36% of the RDA for lactating mothers and thus can be used as sources of zinc.

In the present study, the highest level of manganese was found in FB and FM with values that ranged between 19.4 and 110.6 mg/kg. Lower manganese contents of 3.4 and 4.1 mg/kg in whole Punti and Kachki powders, respectively were reported by Bhowmik et al. (2022). In another study, a relatively high level (6.0 mg/kg) of manganese was reported in Dhaka's fresh Punti (*Puntius sophore*) (Zaman et al., 2014); after the drying process, the manganese content may be close to the value found in the current study due to the enrichment effect. Adequate manganese supplies are considered important for a variety of physiological processes such as 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 adequate intake (AI) of manganese for 14-50-year-old lactating women is 2.6 mg/day (Food and Nutrition Board & Institute of Medicine, 2001c); therefore, 15 g of the DFs in the current study should satisfy at least 6.60% up to 15.08% of this requirement.

3.3.2.2.2 Iron and copper

Iron is the most abundant microelement in the DFs, and the highest content was found in FM with a range of 569.3-194.8 mg/kg while WS had the lowest with 81.6-125.2 mg/kg. Bhowmik et al. (2022) reported a lower iron content (328 mg/kg) in whole Punti powder than 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 (2001a), pregnant women aged 14–30 years have 27 mg/day as the RDA. At least 4.53% up to 47.05% of the RDA of pregnant women can be satisfied by consumption of 15 g of the DFs in present study, which make them excellent sources of dietary iron.

Among all the DFs in the present study, the highest level of copper (80.4 mg/kg) was found in BD-M with FM also having up to 62.0 mg/kg. Bhowmik et al. (2022) reported 5.3 and 3.7 mg/kg of copper in whole Punti and Kachki powders, respectively, which are lower than the values obtained in the present study. Copper is essential as a component of important enzymes that promote cellular respiration, neurotransmitter transmission, and production of peptide hormones to maintain homeostasis (Zhen et al., 2022). Pregnant and lactating women have the highest demand, with RDA values of 1 and 1.3 mg/day (Food and Nutrition Board & Institute of Medicine, 2001a). The DFs (15 g) in this study can meet at least 2.90% up to 93.28% of the RDA of copper and thus can be used as an excellent source of dietary copper.

3.3.2.2.3 Toxicity of excess zinc, iron, manganese, and copper

Since the quantities of microelements needed by the body are limited, it is necessary to consider the toxicity of their high-dose intakes. As mentioned above, iron and zinc were relatively abundant in DFs, which increases the probability of excess intake, especially from frequent consumption. According to the WHO (Codex Alimentarius Commission, 2011), the allowable iron and zinc contents in fish and fish products is 43 and 40 mg/kg respectively, which means that excess consumption can be readily achieved with DFs. According to WHO

(Codex Alimentarius Commission, 2011), the permissible limits of copper and manganese for fish species are 30 and 20 mg/kg, respectively. According to Sousa et al., (2020), excess iron can lead to increased oxidative stress in the body and cause organelle and DNA damage, even cell death. Prolonged excess zinc intake results in copper deficiency and can lead to sideroblastic anemia, granulocytopenia, and myelodysplastic syndrome with neurologic effects (Agnew & Slesinger, 2020). Excess of copper is relatively rare under the context of DFs, except from BD-M, FM-S, and FM-M, which were found to surpass the limit. High copper intake induces Wilson's disease characterized by lethargy, abdominal pain, hepatomegaly, jaundice and other liver symptoms, as well as Indian childhood cirrhosis and idiopathic chronic poisoning (Zhen et al., 2022). In contrast, almost 50% of the DFs in this study had high manganese levels that could lead to excess intake. Consumption of an excessive amount of manganese causes a cumulative effect in the brain that leads to neurotoxicity and consequent neurodegenerative diseases (Martins et al., 2020). Khatun et al. (2021) reported zinc, iron, manganese, copper, etc. exist in large quantities in DFs, which is consistent with results from the present study. The same study also mentioned that seasons and different parts of DFs have significant effects on the microelements content, i.e., the contents in winter are lower than those in summer, and edible parts are lower than those in inedible parts. However, it is worth noting that iron and zinc are the microelements that are mainly deficient within the Bangladesh population (Fiedler et al., 2016), so it is necessary to consider the frequency and method of consumption to estimate the amount of ingested microelements in order to avoid excessive dietary intake.

Table 3. Mineral composition of dried fishes

Sample ID	Phosphorus	Potassium	Calcium	Sodium	Magnesium
	g/100g dried fish				
BD-C	1.21 ± 0.00 ^{f,3}	0.785 ± 0.04 ^{d,2}	1.745 ± 0.02 ^{f,1}	7.39 ± 0.18 ^{d,1}	0.33 ± 0.00 ^{a,1}
BD-D	1.475 ± 0.02 ^{f,1}	1.285 ± 0.04 ^{d,1}	1.665 ± 0.06 ^{f,1}	1.56 ± 0.03 ^{d,2}	0.19 ± 0.00 ^{a,2}
BD-M	1.26 ± 0.00 ^{f,2}	0.655 ± 0.01 ^{d,3}	1.73 ± 0.06 ^{f,1}	1.795 ± 0.01 ^{d,2}	0.24 ± 0.00 ^{a,3}
RF-C	1.75 ± 0.13 ^{e,3}	0.91 ± 0.03 ^{c,3}	2.775 ± 0.45 ^{c,2}	5.99 ± 0.17 ^{e,1}	0.22 ± 0.00 ^{a,2}
RF-D	3.415 ± 0.01 ^{e,1}	1.48 ± 0.01 ^{c,1}	5.695 ± 0.04 ^{c,1}	0.935 ± 0.02 ^{e,3}	0.205 ± 0.01 ^{a,3}
RF-M	2.025 ± 0.06 ^{e,2}	1.255 ± 0.01 ^{c,2}	2.91 ± 0.28 ^{c,2}	2.11 ± 0.03 ^{e,2}	0.29 ± 0.00 ^{a,1}
WS-C	2 ± 0.03 ^{d,2}	1.23 ± 0.03 ^{b,1}	3.165 ± 0.02 ^{d,1}	4.57 ± 0.04 ^{f,1}	0.26 ± 0.01 ^{a,1}
WS-D	2.21 ± 0.01 ^{d,1}	1.595 ± 0.21 ^{b,1}	2.945 ± 0.35 ^{d,1}	1.47 ± 0.08 ^{f,1}	0.27 ± 0.01 ^{a,1}
WS-M	2.235 ± 0.04 ^{d,1}	1.465 ± 0.02 ^{b,1}	3.09 ± 0.37 ^{d,1}	1.27 ± 0.04 ^{f,3}	0.245 ± 0.01 ^{a,1}
FB-S	2.68 ± 0.03 ^{c,1}	0.8 ± 0.00 ^{e,1}	5.58 ± 0.13 ^{b,1}	6.295 ± 0.05 ^{b,1}	0.2 ± 0.00 ^{a,1}
FB-D	2.18 ± 0.01 ^{c,3}	0.725 ± 0.01 ^{e,2}	4.535 ± 0.06 ^{b,3}	3.89 ± 0.03 ^{b,3}	0.15 ± 0.00 ^{a,3}
FB-M	2.245 ± 0.01 ^{c,2}	0.835 ± 0.02 ^{e,1}	4.93 ± 0.13 ^{b,2}	5.765 ± 0.02 ^{b,2}	0.18 ± 0.00 ^{a,2}
GR-S	2.1 ± 0.04 ^{d,2}	1.93 ± 0.31 ^{a,1}	2.155 ± 0.09 ^{e,3}	0.4 ± 0.11 ^{g,1}	0.16 ± 0.01 ^{a,1}
GR-D	2.405 ± 0.04 ^{d,1}	1.75 ± 0.04 ^{a,1}	3.315 ± 0.04 ^{e,1}	0.38 ± 0.00 ^{g,1}	0.175 ± 0.01 ^{a,1}
GR-M	2.175 ± 0.02 ^{d,2}	1.785 ± 0.01 ^{a,1}	2.41 ± 0.03 ^{e,2}	0.445 ± 0.01 ^{g,1}	0.17 ± 0.00 ^{a,1}
FM-S	3.295 ± 0.16 ^{b,1}	0.74 ± 0.04 ^{e,1}	6.54 ± 0.37 ^{a,1}	4.31 ± 0.27 ^{a,2}	0.18 ± 0.00 ^{a,1}
FM-D	2.15 ± 0.00 ^{b,2}	0.675 ± 0.04 ^{e,1}	4.93 ± 0.30 ^{a,2}	7.81 ± 0.24 ^{a,1}	0.2 ± 0.00 ^{a,1}
FM-M	2.23 ± 0.01 ^{b,2}	0.66 ± 0.01 ^{e,1}	4.855 ± 0.04 ^{a,2}	4.76 ± 0.14 ^{a,2}	0.67 ± 0.71 ^{a,1}
FA-S	3.58 ± 0.04 ^{a,1}	0.785 ± 0.01 ^{e,1}	7.005 ± 0.11 ^{a,1}	2.485 ± 0.01 ^{c,3}	0.23 ± 0.00 ^{a,3}
FA-D	2.375 ± 0.01 ^{a,2}	0.72 ± 0.01 ^{e,1}	4.805 ± 0.05 ^{a,2}	3.84 ± 0.04 ^{c,2}	0.26 ± 0.00 ^{a,2}
FA-M	2.35 ± 0.01 ^{a,2}	0.635 ± 0.04 ^{e,2}	5.045 ± 0.15 ^{a,2}	7.21 ± 0.20 ^{c,1}	0.29 ± 0.01 ^{a,1}

Different letters (a, b, c, etc.) represent significant differences between fish types ($p<0.05$). Different numbers (1, 2, and 3) represent significant differences within the same fish type and different locations ($p<0.05$). BD: Bombay duck (*Harpadon nehereus*); RF: ribbon fish (*Trichiurus lepturus*); WS: white sardine (*Escualosa thoracata*); FB: freshwater barb (*Puntius spp.*); GR: Ganges River sprat (*Corica soborna*); FM: fermented barb (*Puntius spp.*); FA: fermented anchovies (*Setipnna spp.*); C: Cox' s Bazar; D: Dhaka; M: Mymensingh; S: Sylhet

Table 3. - *contd.*

Sample ID	Manganese (Mn)	Iron (Fe)	Copper (Cu)	Zinc (Zn)
	mg/kg of dried fish			
BD-C	14.13 ± 0.99 ^{e,1}	194.825 ± 3.67 ^{c,2}	8.605 ± 0.70 ^{b,2}	43.715 ± 4.02 ^{e,1}
BD-D	13.205 ± 0.60 ^{e,12}	307.915 ± 40.64 ^{c,2}	9.26 ± 0.23 ^{b,2}	61.175 ± 5.66 ^{e,1}
BD-M	11.45 ± 0.65 ^{e,2}	415.725 ± 27.32 ^{c,1}	80.845 ± 8.29 ^{b,1}	59.665 ± 14.77 ^{e,1}
RF-C	12.605 ± 0.77 ^{e,1}	84.04 ± 0.91 ^{e,3}	2.31 ± 0.07 ^{c,1}	46.555 ± 0.76 ^{de,1}
RF-D	15.855 ± 4.84 ^{e,1}	174.36 ± 1.88 ^{e,2}	11.48 ± 10.58 ^{c,1}	68.545 ± 13.34 ^{de,1}
RF-M	13.935 ± 0.33 ^{e,1}	261.425 ± 9.16 ^{e,1}	3.33 ± 0.24 ^{c,1}	53.68 ± 11.12 ^{de,1}
WS-C	17.46 ± 0.42 ^{d,2}	81.565 ± 4.45 ^{f,2}	3.835 ± 0.63 ^{c,1}	89.41 ± 7.35 ^{c,1}
WS-D	16.455 ± 0.25 ^{d,3}	125.185 ± 0.70 ^{f,1}	5.88 ± 3.92 ^{c,1}	105.015 ± 9.21 ^{c,1}
WS-M	19.245 ± 0.04 ^{d,1}	86.39 ± 15.78 ^{f,2}	4.15 ± 0.65 ^{c,1}	97.695 ± 1.36 ^{c,1}
FB-S	110.595 ± 2.55 ^{a,1}	200.2 ± 14.92 ^{d,2}	3.465 ± 0.22 ^{c,2}	114.115 ± 2.51 ^{b,2}
FB-D	30.445 ± 0.71 ^{a,2}	248.275 ± 13.30 ^{d,1}	3.665 ± 0.05 ^{c,2}	109.825 ± 0.62 ^{b,2}
FB-M	19.39 ± 0.24 ^{a,3}	229.65 ± 2.56 ^{d,12}	5.76 ± 0.21 ^{c,1}	123.215 ± 0.25 ^{b,1}
GR-S	16.295 ± 1.03 ^{b,3}	202.18 ± 30.66 ^{b,2}	6.37 ± 1.16 ^{c,1}	155.26 ± 12.18 ^{a,2}
GR-D	26.785 ± 0.46 ^{b,2}	174.475 ± 12.48 ^{b,2}	6.555 ± 1.15 ^{c,1}	236.79 ± 0.68 ^{a,1}
GR-M	44.47 ± 3.13 ^{b,1}	846.875 ± 97.25 ^{b,1}	6.875 ± 0.21 ^{c,1}	215.99 ± 6.65 ^{a,1}
FM-S	71.42 ± 0.62 ^{a,1}	194.825 ± 3.69 ^{a,3}	58.845 ± 0.36 ^{a,2}	120.425 ± 1.48 ^{b,1}
FM-D	39.385 ± 0.70 ^{a,3}	343.795 ± 5.45 ^{a,2}	2.945 ± 0.37 ^{a,3}	98.255 ± 3.33 ^{b,3}
FM-M	47.765 ± 0.42 ^{a,2}	569.31 ± 10.11 ^{a,1}	61.985 ± 1.42 ^{a,1}	105.875 ± 1.15 ^{b,2}
FA-S	25.55 ± 0.03 ^{c,2}	187.025 ± 8.58 ^{e,1}	2.515 ± 0.04 ^{c,1}	75.245 ± 0.29 ^{d,1}
FA-D	13.095 ± 0.13 ^{c,3}	143.22 ± 5.61 ^{e,2}	3.195 ± 0.52 ^{c,1}	57.44 ± 0.20 ^{d,3}
FA-M	27.59 ± 0.76 ^{c,1}	197.065 ± 3.02 ^{e,1}	3.45 ± 1.77 ^{c,1}	59.37 ± 0.81 ^{d,2}

Different letters (a, b, c, etc.) represent significant differences between fish types ($p<0.05$). Different numbers (1, 2, and 3) represent significant differences within the same fish type and different locations ($p<0.05$). BD: Bombay duck (*Harpadon nehereus*); RF: ribbon fish (*Trichiurus lepturus*); WS: white sardine (*Escualosa thoracata*); FB: freshwater barb (*Puntius spp.*); GR: Ganges River sprat (*Corica soborna*); FM: fermented barb (*Puntius spp.*); FA: fermented anchovies (*Setipnna spp.*); C: Cox' s Bazar; D: Dhaka; M: Mymensingh; S: Sylhet

3.3.3 Heavy metals

Table 4 shows the content of five heavy metals (arsenic, cadmium, chromium, lead and mercury) in DFs. 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).

Table 4. Heavy metal profile of dried fishes

	Hg	Cr	As	Cd	Pb
	ng/g dried fish		μg/g dried fish		
ML	500 ○	2 §	3.5 ○	0.05▲	0.5○
BD-C	33.58 ± 1.4 ^{f,3}	4.58 ± 3.24 ^{a,1}	1.11 ± 0.03 ^{c,1}	< DL	0.11 ± 0 ^{bc,2}
BD-D	46.99 ± 0.33 ^{f,1}	2.76 ± 0.27 ^{a,1}	1.66 ± 0.02 ^{c,1}	< DL	0.14 ± 0.06 ^{bc,12}
BD-M	37.56 ± 0.02 ^{f,2}	5 ± 1.27 ^{a,1}	1.7 ± 0.37 ^{c,1}	< DL	0.3 ± 0.06 ^{bc,1}
RF-C	85.08 ± 0.02 ^{d,2}	1.19 ± 0.33 ^{b,1}	2.58 ± 0.12 ^{a,3}	< DL	0.04 ± 0.01 ^{cd,2}
RF-D	150.1 ± 13.34 ^{d,1}	0.83 ± 0.07 ^{b,1}	6.2 ± 0.04 ^{a,1}	< DL	0.04 ± 0 ^{cd,2}
RF-M	81.09 ± 3.27 ^{d,2}	1.42 ± 0.03 ^{b,1}	3.14 ± 0.15 ^{a,2}	< DL	0.24 ± 0.01 ^{cd,1}
WS-C	32.98 ± 1.23 ^{f,3}	1.82 ± 1.63 ^{b,1}	3.48 ± 0.07 ^{b,1}	< DL	0.08 ± 0.01 ^{bc,1}
WS-D	40.06 ± 0.17 ^{f,2}	0.61 ± 0.14 ^{b,1}	3.34 ± 0.09 ^{b,1}	< DL	0.4 ± 0.29 ^{bc,1}
WS-M	44.06 ± 0.01 ^{f,1}	0.38 ± 0.46 ^{b,1}	1.93 ± 2.17 ^{b,1}	< DL	0.07 ± 0.07 ^{bc,1}
FB-S	131.41 ± 0.34 ^{b,2}	0.93 ± 0.13 ^{b,2}	0.27 ± 0.03 ^{d,2}	< DL	0.12 ± 0.02 ^{a,3}
FB-D	164.9 ± 6.71 ^{b,1}	2.01 ± 0.54 ^{b,1}	0.21 ± 0.04 ^{d,2}	< DL	0.66 ± 0.02 ^{a,1}
FB-M	117.02 ± 3.58 ^{b,3}	2.87 ± 0.45 ^{b,1}	0.45 ± 0.03 ^{d,1}	< DL	0.2 ± 0.01 ^{a,2}
GR-S	26.22 ± 1.54 ^{e,3}	1.22 ± 0.37 ^{a,1}	0.45 ± 0.01 ^{d,3}	< DL	0.07 ± 0.01 ^{d,1}
GR-D	131.79 ± 1.98 ^{e,1}	4.1 ± 0.09 ^{a,1}	0.48 ± 0.01 ^{d,2}	< DL	0.09 ± 0.02 ^{d,1}
GR-M	42.24 ± 0.69 ^{e,2}	4.41 ± 0.25 ^{a,1}	0.73 ± 0.01 ^{d,1}	< DL	0.09 ± 0.02 ^{d,1}
FM-S	163.34 ± 18.73 ^{a,1}	4.24 ± 0.57 ^{a,1}	0.26 ± 0.02 ^{d,2}	< DL	0.38 ± 0.04 ^{a,1}
FM-D	145.16 ± 2.49 ^{a,1}	1.53 ± 0.05 ^{a,2}	0.35 ± 0.02 ^{d,1}	< DL	0.26 ± 0 ^{a,2}
FM-M	175.93 ± 16.51 ^{a,1}	5.04 ± 0.63 ^{a,1}	0.27 ± 0.03 ^{d,2}	< DL	0.34 ± 0.03 ^{a,12}
FA-S	150.1 ± 9.51 ^{c,1}	1.9 ± 0.5 ^{b,1}	2.96 ± 0.2 ^{b,1}	< DL	0.29 ± 0.01 ^{ab,2}
FA-D	77.03 ± 7.53 ^{c,3}	1.09 ± 0.02 ^{b,1}	3.34 ± 0.25 ^{b,1}	< DL	0.1 ± 0 ^{ab,3}
FA-M	121.1 ± 0.24 ^{c,2}	1.59 ± 0.26 ^{b,1}	2.27 ± 0.07 ^{b,2}	< DL	0.34 ± 0.01 ^{ab,1}

ML: Maximum level of each heavy metal species allowed in fish/fish products (○ refers to Health Canada, 2020; § refers to GB2762-2012, China, 2020; ▲ refers to EC 1881/2006, EU, 2023); BD: Bombay duck (*Harpadon nehereus*); RF: ribbon fish (*Trichiurus iepturus*); WS: white sardine (*Escualosa thoracata*); FB: freshwater barb (*Puntius spp.*); GR: Ganges River sprat (*Corica soborna*); FM: fermented barb (*Puntius spp.*); FA: fermented anchovies (*Setipnna spp.*); C: Cox's Bazar; D: Dhaka; M: Mymensingh; S: Sylhet. Different letters (a, b, and c) represent significant differences between fish types via two-way ANOVA (p<0.05). Different numbers (1, 2, and 3) represent significant differences within the same fish type and different locations via one-way ANOVA (p<0.05).

3.3.3.1 Mercury

Comparing the relevant standard of 500 ng/g, the mercury content in DFs did not exceed the allowable level. Interestingly, a higher level of mercury was found in both FM and FB, which are produced by *Puntius spp.*, while the highest content of 175.93 ± 16.51 ng/g was found in the FM-M. Unfortunately, no relevant reports on the mercury content in these species were found in scientific literature. In another study (Hoque et al., 2022), the mercury content in RF collected from Cox's Bazar was found to be 80 ± 10 ng/g, which is consistent with the results of this study. Mercury is the deadliest heavy metal due to its extremely high affinity for antioxidant molecules (glutathione) which leads to a 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 disease and cerebrovascular accidents (Houston, 2011).

3.3.3.2 Chromium

The excess of chromium the most serious among all heavy metals and contamination comes of water bodies comes mainly from discharged waste products of industries that manufacture paints, plastics, dyes, and inks. It can be seen that BD, FB-D, FB-M, GR-D, GR-M, FM-S and FM-D all exceeded the acceptable standard level. The highest chromium concentration was observed in FM-M and BD-M at 5.04 ± 0.63 and 5 ± 1.27 μg/g, respectively. It is also worth noting that BD, GR, and FM are the three DFs with the highest chromium concentration levels. According to Rakib et al. (2021), the cadmium content of BD collected from Cox’s Bazar is 7.06 ± 0.06 μg/g, which is 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, Bangladesh, and found to contain an average chromium content of as high as 3.824 µg/g. The toxicological mechanism of chromium 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, RF and WS, which have relatively low chromium contents, are more recommended for DF consumption and toxicity prevention than the others.

3.3.3.3 Arsenic

Among all study subjects, only RF-D was found to contain excess arsenic of up to 6.2 ± 0.04 µg/g. In a previous study (Kar et al., 2020), it was found that the arsenic content of RF collected from Cox's Bazar was 22.27 µg/g, which is much higher than the present finding. Inorganic arsenic, as a group-1 carcinogen, can inactivate up to 200 enzymes, especially those involved in cellular energy pathways and 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) mentioned that organic arsenic is not toxic and tends to accumulate in fish, this does not endorse the safety of DFs with high arsenic content. The main reason is that during the production and preservation processes of DFs, unscrupulous merchants will spray pesticides on dried fish, which may contain carcinogenic inorganic arsenic (Kar et al., 2020; Khatun et al., 2021). Therefore, it is recommended to consume DFs that contain arsenic within the restricted threshold.

3.3.3.4 Cadmium and lead

The cadmium contents of all samples in this study are below the detection value. This finding is consistent with the results reported in many previous publications (Kar et al., 2020; Khatun et al., 2021). Cadmium poisoning can induce chronic diseases as well as damages to internal organs and the reproductive system (Genchi et al., 2020). Industrial pollution is the main source of cadmium contamination, and cadmium is relatively enriched in rice, vegetables, and shellfish, so the low cadmium content of DFs used in this study is desirable (Genchi et al., 2020). In this study, it was found that the lead content of all DFs except FB-D (0.66 ± 0.02 μg/g) was below the corresponding allowed maximum level (ML). Bhowmik et al. (2022) reported that the lead content of whole Punti powder was 0.47 ± 0.01 μg/g, which is slightly lower than the level found in this study. In addition, it was found that FB and FM, two DFs with higher fat content produced from the same species (*Puntius spp.*), have the highest lead content, which may be explained by the high oil affinity of lead (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 about consuming DFs with high lead content.

3.3.4 Amino acid composition and digestible essential amino acid score (DEAAS)

Table 5 shows the percentage amino acid composition of DFs, presented as mean ± standard deviation. Amino acids are the basic component of proteins, which are linearly linked by peptide bonds, and are vital in nutrition and metabolism in the human body. According to the ability of the human body to synthesize sufficient physiological requirements, amino acids can be divided into essential (EAA) and non-essential (NAA). The EAA include histidine (His),

arginine (Arg), threonine (Thr), valine (Val), methionine (Met), Isoleucine (Ile), leucine (Leu), phenylalanine (Phe), tryptophan (Trp), and lysine (Lys). NAA are alanine (Ala), aspartic acid (Asp), asparagine (Asn), cystine (Cys), glutamic acid (Glu), glutamine (Gln), glycine (Gly), proline (Pro), serine (Ser), and tyrosine (Tyr) (Wu, 2009). Table 5 also includes the DEAAS, which was calculated according to the FAO Expert Consultation (2011).

The predominant amino acids in DFs are glutamic acid + glutamine, followed by aspartic acid + asparagine, lysine and leucine, accounting for 16.89-13.71, 10.41-7.97, 9.12-6.93, and 8.36-7.36 % of the total amino acids, respectively. 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 the normal functioning of the immune system and intestines (David, 2012a, 2012b; Li et al., 2007). Shah et al. (2020) summarized that glutamine is key to the amino acid metabolism and is thought to be the fuel for regular activities of the immune system. Asparagine is recognized for its ability to prevent lymphocyte apoptosis and promote an immune response (Li et al., 2007). Lysine, as one of the EAA, is the most commonly deficient amino acid in developing countries where cereals are consumed as the staple food (Yang et al., 2022). It is very important for human health, via maintaining the immune system, building the structural proteins of connective tissue, and controlling the fatty acid metabolism (Yang et al., 2022). Leucine is a promoter of cell growth and division, an anabolic mediator of protein metabolism, and has showed positive impact on the muscle protein synthesis, which can help to maintain healthy amounts of muscle (Beaudry & Law, 2022). In addition, in this study, a relatively abundant arginine was found in DFs protein, which accounts for about 4.59-6.98%

of the total amino acid. Arginine is a substrate for nitric oxide production, especially when the human body is faced with pathogenic pressure. In addition, the amount of arginine that is synthesized by the body may not be able to meet the needs of adults and children, thus additional exogenous intake is necessary (Li et al., 2007; Wu et al., 2021).

It was found that the EAA proportion of most DFs in this study is less than 50% of the total amino acids. However, there are exceptions, such as GR and WS whose EAA contents are significantly higher than those of other DFs, and reaching maximum values of 50.96% and 51.26%, respectively. 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). Because of the role of EAA, which is to maintain good health and normal body functions, external EAA intake is necessary. Therefore, the DFs in this study, especially WS and GR, can serve as good sources of EAA supplementation for the human body. It is also worth noting that the content of branched-chain amino acids (BCAA), namely leucine, isoleucine, and valine, account for about 16.79-18.66% of the total amino acids, which is consistent with the approximately 15% previously reported value (Usydus et al., 2009). Similar to 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 the body vulnerability to bacteria and virus infections (Li et al., 2007). Bassit et al. (2002) reported that adding 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.

Although, in present study, the EAA content in most DFs is less than 50% of the total AA,

it should not be a sufficient reason for consumers to reject DFs. 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 (that is, 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 limit of DFs protein, which can better meet the human body's demand for EAA (FAO Expert Consultation, 2011). As shown in Table 5, the DEAAS of DFs, except tryptophan (95%) of FM-D and histidine (99%) of FA-D, are all higher than 100%. The results indicate that DFs in the present study, in terms of EAA quality, can meet ≥95% of the adult human’s EAA requirement, and therefore can be considered as excellent source of high-quality protein.

Table 5. Percentage amino acid composition of dried fishes (g/100 g of protein)

	BD-C	BD-D	BD-M	RF-C	RF-D
His	1.91 ± 0.13	2.41 ± 0.07	2.13 ± 0.29	2.28 ± 0.01	2.43 ± 0.13
Arg	6.00 ± 0.04	6.06 ± 0.07	6.80 ± 0.22	6.46 ± 0.17	6.50 ± 0.08
Thr	4.47 ± 0.07	4.18 ± 0.02	4.46 ± 0.13	4.53 ± 0.06	4.46 ± 0.05
Val	5.37 ± 0.11	5.18 ± 0.07	5.30 ± 0.16	5.15 ± 0.06	5.14 ± 0.09
Met	3.18 ± 0.22	4.70 ± 0.05	3.82 ± 0.27	3.29 ± 0.05	3.83 ± 0.08
Ile	4.77 ± 0.11	4.58 ± 0.03	4.74 ± 0.24	4.81 ± 0.13	4.92 ± 0.09
Leu	8.26 ± 0.22	7.64 ± 0.12	8.23 ± 0.39	7.81 ± 0.26	7.95 ± 0.27
Phe	4.41 ± 0.14	4.12 ± 0.04	4.33 ± 0.25	4.42 ± 0.07	4.48 ± 0.22
Trp	1.05 ± 0.03	1.26 ± 0.01	1.12 ± 0.04	1.00 ± 0.25	1.17 ± 0.24
Lys	8.53 ± 0.21	7.76 ± 0.09	8.59 ± 0.01	8.78 ± 0.15	8.64 ± 0.13
BCAA	18.39 ± 0.44 ^{ab,1}	17.40 ± 0.22 ^{ab,1}	18.26 ± 0.79 ^{ab,1}	17.76 ± 0.45 ^{ab,1}	18.00 ± 0.44 ^{ab,1}
EAA	47.93 ± 0.78 ^{bc,1}	47.88 ± 0.13 ^{bc,1}	49.52 ± 0.87 ^{bc,1}	48.50 ± 0.38 ^{b,1}	49.52 ± 0.95 ^{b,1}
Ala	6.41 ± 0.15	6.60 ± 0.02	6.29 ± 0.18	6.30 ± 0.14	6.07 ± 0.18
Asx	9.13 ± 0.45	9.70 ± 0.04	9.28 ± 0.47	10.01 ± 0.10	9.52 ± 0.36
Cys	0.82 ± 0.06	0.92 ± 0.00	0.78 ± 0.04	0.89 ± 0.05	1.10 ± 0.02
Glx	16.10 ± 0.28	15.58 ± 0.04	15.82 ± 0.63	15.42 ± 0.20	15.18 ± 0.36
Gly	6.62 ± 0.03	6.52 ± 0.20	6.09 ± 0.04	6.64 ± 0.58	6.27 ± 0.15
Pro	4.83 ± 0.06	4.65 ± 0.03	4.53 ± 0.03	4.59 ± 0.28	4.36 ± 0.07
Ser	3.73 ± 0.01	3.67 ± 0.09	3.83 ± 0.11	4.02 ± 0.09	3.97 ± 0.10
Tyr	4.43 ± 0.13	4.47 ± 0.13	3.86 ± 0.35	3.62 ± 0.18	4.03 ± 0.05
DEAAS (%)	127 (His)	129 (Leu)	136 (Val)	132 (Val)	132 (Leu, Val)

BD: Bombay duck (*Harpadon nehereus*); RF: ribbon fish (*Trichiurus iepturus*); WS: white sardine (*Escualosa thoracata*); FB: freshwater barb (*Puntius spp.*); GR: Ganges River sprat (*Corica soborna*); FM: fermented barb (*Puntius spp.*); FA: fermented anchovies (*Setipnna spp.*); C: Cox's Bazar; D: Dhaka; M: Mymensingh; S: Sylhet. Different letters (a, b, and c) represent significant differences between fish types via two-way ANOVA (p<0.05). Different numbers (1, 2, and 3) represent significant differences within the same fish type and different locations via one-way ANOVA (p<0.05); His: histidine; Arg: arginine; Thr : threonine; Val: valine; Met: methionine; Ile: isoleucine; Leu: leucine; Phe: phenylalanine; Trp: tryptophan; Lys: lysine; Ala: alanine; Asx- Aspartic acid +asparagine; Cys: cysteine; Glx- Glutamic acid +glutamine; Gly: glycine; Pro: proline; Ser: serine; Tyr; tyrosine; BCAA- Branched-chain amino acids- leucine, isoleucine, and valine; EAA- Essential amino acids- histidine, arginine, threonine, valine, methionine, isoleucine, leucine, phenylalanine, tryptophan, and lysine; DEAAS (%)- Digestible EAA score (%) = 100 x lowest value ["Digestible EAA reference ratio" for a given amino acid scoring pattern (FAO Expert Consultation, 2011); Data present as value of DEAAS and (the limiting AA); Detailed AA scores (%) of various EAA are shown in Supplementary Table 1.

Table 5. - *contd.*

	RF-M	WS-C	WS-D	WS-M
His	2.06 ± 0.12	3.33 ± 0.37	2.79 ± 0.07	2.55 ± 0.13
Arg	6.98 ± 0.08	6.01 ± 0.19	6.39 ± 0.10	6.49 ± 0.07
Thr	4.54 ± 0.06	4.59 ± 0.07	4.53 ± 0.03	4.45 ± 0.04
Val	5.19 ± 0.07	5.43 ± 0.13	5.39 ± 0.02	5.31 ± 0.05
Met	3.04 ± 0.17	3.70 ± 0.08	3.38 ± 0.04	3.88 ± 0.37
Ile	4.97 ± 0.16	4.92 ± 0.16	4.84 ± 0.00	4.84 ± 0.07
Leu	8.36 ± 0.17	8.31 ± 0.21	8.33 ± 0.02	8.34 ± 0.01
Phe	4.56 ± 0.07	4.82 ± 0.25	4.74 ± 0.06	4.79 ± 0.00
Trp	0.87 ± 0.06	1.57 ± 0.05	1.38 ± 0.03	1.49 ± 0.12
Lys	9.09 ± 0.31	8.35 ± 0.15	9.02 ± 0.11	9.12 ± 0.13
BCAA	18.51 ± 0.40 ^{ab,1}	18.66 ± 0.51 ^{a,1}	18.56 ± 0.00 ^{a,1}	18.49 ± 0.11 ^{a,1}
EAA	49.66 ± 0.87 ^{b,1}	51.03 ± 0.67 ^{a,1}	50.78 ± 0.01 ^{a,1}	51.26 ± 0.27 ^{a,1}
Ala	5.82 ± 0.21	6.15 ± 0.00	6.06 ± 0.01	6.01 ± 0.05
Asx	10.41 ± 0.15	9.37 ± 0.46	9.97 ± 0.10	9.82 ± 0.14
Cys	0.82 ± 0.03	1.15 ± 0.03	1.02 ± 0.05	1.09 ± 0.08
Glx	15.59 ± 0.23	14.93 ± 0.51	15.18 ± 0.09	15.14 ± 0.13
Gly	5.60 ± 0.69	5.45 ± 0.14	5.13 ± 0.04	5.05 ± 0.12
Pro	4.17 ± 0.29	4.15 ± 0.10	3.97 ± 0.04	3.96 ± 0.04
Ser	4.15 ± 0.08	3.96 ± 0.06	3.94 ± 0.09	3.84 ± 0.02
Tyr	3.78 ± 0.12	3.82 ± 0.03	3.94 ± 0.16	3.84 ± 0.17
DEAAS (%)	132 (Val)	139 (Val)	138 (Val)	136 (Val)

BD: Bombay duck (*Harpadon nehereus*); RF: ribbon fish (*Trichiurus iepturus*); WS: white sardine (*Escualosa thoracata*); FB: freshwater barb (*Puntius spp.*); GR: Ganges River sprat (*Corica soborna*); FM: fermented barb (*Puntius spp.*); FA: fermented anchovies (*Setipnna spp.*); C: Cox's Bazar; D: Dhaka; M: Mymensingh; S: Sylhet. Different letters (a, b, and c) represent significant differences between fish types via two-way ANOVA (p<0.05). Different numbers (1, 2, and 3) represent significant differences within the same fish type and different locations via one-way ANOVA (p<0.05); His: histidine; Arg: arginine; Thr : threonine; Val: valine; Met: methionine; Ile: isoleucine; Leu: leucine; Phe: phenylalanine; Trp: tryptophan; Lys: lysine; Ala: alanine; Asx- Aspartic acid +asparagine; Cys: cysteine; Glx- Glutamic acid +glutamine; Gly: glycine; Pro: proline; Ser: serine; Tyr; tyrosine; BCAA- Branched-chain amino acids- leucine, isoleucine, and valine; EAA- Essential amino acids- histidine, arginine, threonine, valine, methionine, isoleucine, leucine, phenylalanine, tryptophan, and lysine; DEAAS (%)- Digestible EAA score (%) = 100 x lowest value ["Digestible EAA reference ratio" for a given amino acid scoring pattern (FAO Expert Consultation, 2011); Data present as value of DEAAS and (the limiting AA); Detailed AA scores (%) of various EAA are shown in Supplementary Table 1.

Table 5. - *contd.*

	FB-S	FB-D	FB-M	GR-S	GR-D	GR-M
His	2.62 ± 0.20	2.63 ± 0.19	2.36 ± 0.01	3.56 ± 0.16	2.78 ± 0.18	3.16 ± 0.31
Arg	6.02 ± 0.02	6.38 ± 0.11	6.76 ± 0.43	6.75 ± 0.05	6.65 ± 0.02	6.87 ± 0.06
Thr	4.40 ± 0.14	4.31 ± 0.06	4.40 ± 0.09	4.47 ± 0.02	4.50 ± 0.01	4.56 ± 0.07
Val	5.20 ± 0.20	5.09 ± 0.08	5.09 ± 0.10	5.33 ± 0.01	5.35 ± 0.01	5.40 ± 0.02
Met	2.85 ± 0.18	2.98 ± 0.15	2.70 ± 0.01	3.51 ± 0.09	3.27 ± 0.12	3.11 ± 0.12
Ile	4.71 ± 0.17	4.55 ± 0.04	4.63 ± 0.09	4.71 ± 0.02	4.72 ± 0.02	4.83 ± 0.05
Leu	7.97 ± 0.30	7.90 ± 0.08	7.97 ± 0.12	8.03 ± 0.07	8.17 ± 0.03	8.04 ± 0.03
Phe	4.84 ± 0.14	4.91 ± 0.16	5.06 ± 0.27	4.72 ± 0.03	4.71 ± 0.00	4.80 ± 0.00
Trp	0.81 ± 0.02	0.91 ± 0.11	0.69 ± 0.15	1.19 ± 0.03	1.11 ± 0.04	1.17 ± 0.01
Lys	8.27 ± 0.39	8.50 ± 0.21	8.63 ± 0.41	8.70 ± 0.18	8.99 ± 0.03	8.76 ± 0.26
BCAA	17.88 ± 0.67 ^{bc,1}	17.53 ± 0.12 ^{bc,1}	17.69 ± 0.32 ^{bc,1}	18.06 ± 0.11 ^{ab,1}	18.24 ± 0.01 ^{ab,1}	18.27 ± 0.06 ^{ab,1}
EAA	47.69 ± 1.31 ^{c,1}	48.17 ± 0.11 ^{c,1}	48.29 ± 0.55 ^{c,1}	50.96 ± 0.10 ^{a,1}	50.26 ± 0.28 ^{a,2}	50.72 ± 0.11 ^{a,12}
Ala	6.44 ± 0.10	6.31 ± 0.02	6.24 ± 0.06	5.80 ± 0.04	5.96 ± 0.10	5.81 ± 0.04
Asx	9.69 ± 0.37	9.91 ± 0.42	9.82 ± 0.90	9.86 ± 0.01	10.01 ± 0.07	10.03 ± 0.17
Cys	0.88 ± 0.11	0.89 ± 0.09	0.82 ± 0.02	1.05 ± 0.04	1.05 ± 0.01	0.99 ± 0.05
Glx	14.96 ± 0.30	14.98 ± 0.44	14.63 ± 0.87	14.80 ± 0.07	15.14 ± 0.07	14.61 ± 0.12
Gly	7.30 ± 0.44	7.07 ± 0.31	7.32 ± 0.71	5.12 ± 0.09	5.39 ± 0.25	5.39 ± 0.11
Pro	5.03 ± 0.19	4.82 ± 0.19	4.94 ± 0.38	4.18 ± 0.04	4.27 ± 0.16	4.23 ± 0.01
Ser	4.23 ± 0.04	4.10 ± 0.10	4.25 ± 0.15	4.16 ± 0.00	4.14 ± 0.04	4.25 ± 0.10
Tyr	3.79 ± 0.02	3.75 ± 0.08	3.69 ± 0.03	4.07 ± 0.04	3.78 ± 0.05	3.98 ± 0.14
DEAAS (%)	133 (Val)	130 (Val)	131 (Val)	136 (Leu)	137 (Val)	136 (Leu)

BD: Bombay duck (*Harpadon nehereus*); RF: ribbon fish (*Trichiurus iepturus*); WS: white sardine (*Escualosa thoracata*); FB: freshwater barb (*Puntius spp.*); GR: Ganges River sprat (*Corica soborna*); FM: fermented barb (*Puntius spp.*); FA: fermented anchovies (*Setipnna spp.*); C: Cox's Bazar; D: Dhaka; M: Mymensingh; S: Sylhet. Different letters (a, b, and c) represent significant differences between fish types via two-way ANOVA (p<0.05). Different numbers (1, 2, and 3) represent significant differences within the same fish type and different locations via one-way ANOVA (p<0.05); His: histidine; Arg: arginine; Thr : threonine; Val: valine; Met: methionine; Ile: isoleucine; Leu: leucine; Phe: phenylalanine; Trp: tryptophan; Lys: lysine; Ala: alanine; Asx- Aspartic acid +asparagine; Cys: cysteine; Glx- Glutamic acid +glutamine; Gly: glycine; Pro: proline; Ser: serine; Tyr; tyrosine; BCAA- Branched-chain amino acids- leucine, isoleucine, and valine; EAA- Essential amino acids- histidine, arginine, threonine, valine, methionine, isoleucine, leucine, phenylalanine, tryptophan, and lysine; DEAAS (%)- Digestible EAA score (%) = 100 x lowest value ["Digestible EAA reference ratio" for a given amino acid scoring pattern (FAO Expert Consultation, 2011); Data present as value of DEAAS and (the limiting AA); Detailed AA scores (%) of various EAA are shown in Supplementary Table 1.

Table 5. - *contd.*

	FM-S	FM-D	FM-M	FA-S	FA-D	FA-M
His	2.89 ± 0.27	2.22 ± 0.02	2.84 ± 0.16	1.61 ± 0.27	1.48 ± 0.03	2.23 ± 0.12
Arg	5.31 ± 0.35	6.73 ± 0.27	4.85 ± 0.18	5.01 ± 0.37	4.59 ± 0.29	4.92 ± 0.06
Thr	3.27 ± 0.05	4.08 ± 0.05	2.90 ± 0.00	2.87 ± 0.03	3.64 ± 0.12	3.98 ± 0.03
Val	5.02 ± 0.13	5.03 ± 0.04	5.29 ± 0.07	5.13 ± 0.28	5.36 ± 0.25	5.32 ± 0.02
Met	3.16 ± 0.10	2.60 ± 0.10	3.14 ± 0.44	4.87 ± 0.08	3.50 ± 0.21	3.00 ± 0.02
Ile	4.41 ± 0.16	4.48 ± 0.04	4.63 ± 0.19	4.41 ± 0.35	4.84 ± 0.44	4.86 ± 0.06
Leu	7.36 ± 0.36	7.62 ± 0.10	7.54 ± 0.28	7.36 ± 0.59	7.81 ± 0.52	7.76 ± 0.12
Phe	4.29 ± 0.07	5.22 ± 0.08	4.70 ± 0.12	4.34 ± 0.24	5.01 ± 0.24	4.95 ± 0.07
Trp	0.64 ± 0.00	0.57 ± 0.08	0.71 ± 0.14	0.76 ± 0.20	0.88 ± 0.21	1.02 ± 0.04
Lys	7.55 ± 0.57	7.41 ± 0.16	8.82 ± 0.10	6.93 ± 0.61	8.19 ± 0.51	8.67 ± 0.05
BCAA	16.79 ± 0.64 ^{c,1}	17.14 ± 0.10 ^{c,1}	17.45 ± 0.54 ^{c,1}	16.89 ± 1.22 ^{bc,1}	18.01 ± 1.21 ^{bc,1}	17.95 ± 0.20 ^{bc,1}
EAA	43.89 ± 1.35 ^{d,2}	45.98 ± 0.19 ^{d,1}	45.40 ± 0.11 ^{d,1}	43.27 ± 1.88 ^{d,1}	45.29 ± 1.82 ^{d,1}	46.72 ± 0.31 ^{d,1}
Ala	7.82 ± 0.15	6.58 ± 0.01	9.42 ± 0.03	7.96 ± 0.39	7.87 ± 0.33	6.50 ± 0.04
Asx	9.46 ± 0.08	9.08 ± 1.04	7.97 ± 0.20	8.56 ± 0.19	8.93 ± 0.19	10.37 ± 0.03
Cys	0.83 ± 0.11	0.73 ± 0.05	0.84 ± 0.06	0.85 ± 0.13	0.70 ± 0.02	0.90 ± 0.03
Glx	14.89 ± 0.17	13.71 ± 1.02	16.15 ± 0.05	15.87 ± 0.63	16.43 ± 0.36	16.89 ± 0.04
Gly	10.04 ± 1.11	9.81 ± 1.33	8.69 ± 0.23	10.30 ± 1.76	8.10 ± 1.44	6.55 ± 0.23
Pro	6.54 ± 0.58	6.19 ± 0.69	5.76 ± 0.12	6.93 ± 0.85	5.54 ± 0.70	4.59 ± 0.15
Ser	3.36 ± 0.06	4.01 ± 0.02	2.98 ± 0.03	2.66 ± 0.09	3.07 ± 0.07	3.28 ± 0.00
Tyr	3.18 ± 0.20	3.91 ± 0.07	2.78 ± 0.05	3.60 ± 0.52	4.09 ± 0.04	4.20 ± 0.14
DEAAS (%)	106 (Trp)	95 (Trp)	118 (Trp)	108 (His)	99 (His)	132 (leu)

BD: Bombay duck (*Harpadon nehereus*); RF: ribbon fish (*Trichiurus iepturus*); WS: white sardine (*Escualosa thoracata*); FB: freshwater barb (*Puntius spp.*); GR: Ganges River sprat (*Corica soborna*); FM: fermented barb (*Puntius spp.*); FA: fermented anchovies (*Setipnna spp.*); C: Cox's Bazar; D: Dhaka; M: Mymensingh; S: Sylhet. Different letters (a, b, and c) represent significant differences between fish types via two-way ANOVA ($p<0.05$). Different numbers (1, 2, and 3) represent significant differences within the same fish type and different locations via one-way ANOVA ($p<0.05$); His: histidine; Arg: arginine; Thr : threonine; Val: valine; Met: methionine; Ile: isoleucine; Leu: leucine; Phe: phenylalanine; Trp: tryptophan; Lys: lysine; Ala: alanine; Asx- Aspartic acid +asparagine; Cys: cysteine; Glx- Glutamic acid +glutamine; Gly: glycine; Pro: proline; Ser: serine; Tyr; tyrosine; BCAA- Branched-chain amino acids- leucine, isoleucine, and valine; EAA- Essential amino acids- histidine, arginine, threonine, valine, methionine, isoleucine, leucine, phenylalanine, tryptophan, and lysine; DEAAS (%)- Digestible EAA score (%) = 100 x lowest value ["Digestible EAA reference ratio" for a given amino acid scoring pattern (FAO Expert Consultation, 2011); Data present as value of DEAAS and (the limiting AA); Detailed AA scores (%) of various EAA are shown in Supplementary Table 1.

Supplementary Table 1. Amino Acid Score (%)

Sample	His	Ile	Leu	Lys	Met+Cys	Phe+Tyr	Thr	Trp	Val
BD-C	127 ^{d,1}	159 ^{abc,1}	140 ^{ab,1}	189 ^{b,1}	182 ^{a,2}	233 ^{ab,1}	194 ^{b,1}	174 ^{b,2}	138 ^{ab,1}
BD-D	161 ^{d,1}	153 ^{abc,1}	129 ^{ab,1}	172 ^{b,2}	255 ^{a,1}	226 ^{ab,1}	182 ^{b,2}	209 ^{b,1}	133 ^{ab,1}
BD-M	142 ^{d,1}	158 ^{abc,1}	139 ^{ab,1}	191 ^{b,1}	209 ^{a,2}	216 ^{ab,1}	194 ^{b,1}	187 ^{b,2}	136 ^{ab,1}
RF-C	152 ^{d,12}	160 ^{a,1}	132 ^{ab,1}	195 ^{a,1}	190 ^{b,2}	211 ^{bc,1}	197 ^{a,1}	166 ^{bc,1}	132 ^{bcd,1}
RF-D	162 ^{d,1}	164 ^{a,1}	135 ^{ab,1}	192 ^{a,1}	224 ^{b,1}	224 ^{bc,1}	194 ^{a,1}	195 ^{bc,1}	132 ^{bcd,1}
RF-M	137 ^{d,2}	166 ^{a,1}	142 ^{ab,1}	202 ^{a,1}	175 ^{b,2}	219 ^{bc,1}	197 ^{a,1}	145 ^{bc,1}	133 ^{bcd,1}
WS-C	222 ^{b,1}	164 ^{a,1}	141 ^{a,1}	186 ^{a,2}	220 ^{a,1}	227 ^{ab,1}	200 ^{a,1}	261 ^{a,1}	139 ^{a,1}
WS-D	186 ^{b,12}	161 ^{a,1}	141 ^{a,1}	200 ^{a,1}	200 ^{a,1}	228 ^{ab,1}	197 ^{a,1}	229 ^{a,1}	138 ^{a,1}
WS-M	170 ^{b,2}	161 ^{a,1}	141 ^{a,1}	203 ^{a,1}	226 ^{a,1}	227 ^{ab,1}	193 ^{a,1}	248 ^{a,1}	136 ^{a,1}
FB-S	175 ^{c,1}	157 ^{bc,1}	135 ^{bc,1}	184 ^{ab,1}	170 ^{c,1}	227 ^{a,1}	191 ^{b,1}	135 ^{d,1}	133 ^{cd,1}
FB-D	176 ^{c,1}	152 ^{bc,1}	134 ^{bc,1}	189 ^{ab,1}	176 ^{c,1}	228 ^{a,1}	188 ^{b,1}	152 ^{d,1}	130 ^{cd,1}
FB-M	157 ^{c,1}	154 ^{bc,1}	135 ^{bc,1}	192 ^{ab,1}	160 ^{c,1}	230 ^{a,1}	191 ^{b,1}	114 ^{d,1}	131 ^{cd,1}
GR-S	237 ^{a,1}	157 ^{ab,2}	136 ^{ab,1}	193 ^{a,1}	207 ^{b,1}	231 ^{a,1}	194 ^{a,1}	198 ^{b,1}	137 ^{a,2}
GR-D	185 ^{a,2}	157 ^{ab,12}	139 ^{ab,1}	200 ^{a,1}	196 ^{b,12}	223 ^{a,1}	195 ^{a,1}	186 ^{b,1}	137 ^{a,2}
GR-M	211 ^{a,12}	161 ^{ab,1}	136 ^{ab,1}	195 ^{a,1}	187 ^{b,2}	231 ^{a,1}	198 ^{a,1}	196 ^{b,1}	138 ^{a,1}
FM-S	193 ^{c,1}	147 ^{c,1}	125 ^{d,1}	168 ^{c,2}	182 ^{c,1}	196 ^{c,2}	142 ^{c,2}	106 ^{e,1}	129 ^{d,2}
FM-D	148 ^{c,2}	149 ^{c,1}	129 ^{d,1}	165 ^{c,2}	151 ^{c,1}	240 ^{c,1}	178 ^{c,1}	95 ^{e,1}	129 ^{d,12}
FM-M	189 ^{c,12}	154 ^{c,1}	128 ^{d,1}	196 ^{c,1}	181 ^{c,1}	197 ^{c,2}	126 ^{c,3}	118 ^{e,1}	136 ^{d,1}
FA-S	108 ^{e,2}	147 ^{abc,1}	125 ^{cd,1}	154 ^{c,2}	260 ^{a,1}	209 ^{a,1}	125 ^{c,3}	126 ^{cd,1}	132 ^{abc,1}
FA-D	99 ^{e,2}	161 ^{abc,1}	132 ^{cd,1}	182 ^{c,12}	191 ^{a,12}	239 ^{a,1}	158 ^{c,2}	146 ^{cd,1}	137 ^{abc,1}
FA-M	149 ^{e,1}	162 ^{abc,1}	132 ^{cd,1}	193 ^{c,1}	177 ^{a,12}	241 ^{a,1}	173 ^{c,1}	170 ^{cd,1}	136 ^{abc,1}

BD: Bombay duck (*Harpadon nehereus*); RF: ribbon fish (*Trichiurus iepturus*); WS: white sardine (*Escualosa thoracata*); FB: freshwater barb (*Puntius spp.*); GR: Ganges River sprat (*Corica soborna*); FM: fermented barb (*Puntius spp.*); FA: fermented anchovies (*Setipnna spp.*); C: Cox's Bazar; D: Dhaka; M: Mymensingh; S: Sylhet. Amino acid score is calculated with essential amino acid requirements for adults according to amino acid scoring pattern (FAO Expert Consultation, 2011). His: histidine; Ile: isoleucine; Leu: leucine; Lys: lysine; Met: methionine; Cys: cysteine; Phe: phenylalanine; Tyr; tyrosine; Thr: threonine; Trp: tryptophan; Val: valine. Different letters (a, b, and c) represent significant differences between fish types via two-way ANOVA (p<0.05). Different numbers (1, 2, and 3) represent significant differences within the same

3.3.5 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 the 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 being for RF (85.3 ± 0.64 - 83.22 ± 0.26%) followed by WS with a range of 80.5 ± 0.26 - 79.51 ± 0.13% (Table 6). However, GR, which exhibited an outstanding content of amino acids, had IVPD in the 77.97 ± 0 - 77.15 ± 0.13% range, which ranked the lowest of all the DFs (except for fermented FB and FA). In general, the IVPD results of DFs in present study are consistent with or higher than the 72% reported for freshly filleted *Thichiurus lepturus* (Ribbon fish, 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 include salting and drying, which are inevitable in DFs making may lead to adverse structure changes in the protein due to oxygenation; e.g., 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 *Thichiurus lepturus* (Ribbon fish, RF), and found that cooking methods of all kinds significantly increased digestibility more than the values obtained for uncooked

Table 6. Vitamin B12 content, cholesterol content, protein composition and in vitro digestibility of dried fishes

Sample ID	VB_{12} Content (ug/g)	Cholesterol content (mg/g)	Sarcoplasmic protein Yields (%)	Myofibrilla protein Yields (%)	Sarcoplasmic /Myofibrillar Ratio	Percent digestibility (Y%)
BD-C	0.031 ± 0.001 ^{e}	5.129 ± 0.146 ^{c,2}	18.75 ± 0.1 ^{e,1}	2.75 ± 0 ^{g,1}	6.81:1 ^{a,1}	80.59 ± 0.64 ^{c,1}
BD-D	0.077 ± 0.003 ^{e}	5.809 ± 0.066 ^{c,1}	10.11 ± 0.1 ^{e,2}	2.1 ± 0.09 ^{g,2}	4.81:1 ^{a,2}	76.16 ± 0 ^{c,2}
BD-M	N/A	5.978 ± 0.135 ^{c,1}	6.82 ± 0.16 ^{e,3}	2.1 ± 0.02 ^{g,2}	3.25:1 ^{a,3}	79.96 ± 0.26 ^{c,1}
RF-C	0.056 ± 0.001 ^{b,2}	4.946 ± 0.308 ^{c,3}	10.17 ± 0.09 ^{g,1}	4.11 ± 0.09 ^{f,1}	2.47:1 ^{e,1}	83.22 ± 0.26 ^{a,2}
RF-D	0.149 ± 0.008 ^{b,1}	6.645 ± 0.150 ^{c,1}	6.66 ± 0.02 ^{g,2}	3.76 ± 0.23 ^{f,1}	1.78:1 ^{e,2}	83.49 ± 0.38 ^{a,2}
RF-M	0.051 ± 0.000 ^{b,2}	5.588 ± 0.085 ^{c,2}	3.83 ± 0.01 ^{g,3}	2.59 ± 0.04 ^{f,2}	1.48:1 ^{e,3}	85.3 ± 0.64 ^{a,1}
WS-C	0.081 ± 0.001 ^{c,1}	7.473 ± 0.010 ^{b,2}	20.62 ± 0.13 ^{b,1}	10.61 ± 0.1 ^{a,2}	1.94:1 ^{f,1}	79.51 ± 0.13 ^{b,2}
WS-D	0.072 ± 0.003 ^{c,12}	8.402 ± 0.235 ^{b,1}	15.6 ± 0.04 ^{b,2}	11.38 ± 0.12	1.37:1 ^{f,3}	79.6 ± 0 ^{b,2}
WS-M	0.066 ± 0.011 ^{c,2}	8.836 ± 0.044 ^{b,1}	13.82 ± 0.11 ^{b,3}	9.29 ± 0.19 ^{a,3}	1.49:1 ^{f,2}	80.5 ± 0.26 ^{b,1}
FB-S	0.063 ± 0.006 ^{d,1}	5.636 ± 0.204 ^{c,1}	14.04 ± 0.02 ^{d,2}	6.46 ± 0.16 ^{d,2}	2.17:1 ^{d,1}	77.61 ± 0.26 ^{d,2}
FB-D	0.061 ± 0.002 ^{d,1}	5.943 ± 0.607 ^{c,1}	16.06 ± 0.28 ^{d,1}	7.63 ± 0.03 ^{d,1}	2.11:1 ^{d,1}	76.7 ± 0.51 ^{d,2}
FB-M	0.064 ± 0.004 ^{d,1}	5.634 ± 0.098 ^{c,1}	8.84 ± 0.11 ^{d,3}	4.24 ± 0.23 ^{d,3}	2.09:1 ^{d,1}	80.41 ± 0.13 ^{d,1}
GR-S	0.203 ± 0.005 ^{a,2}	10.495 ± 0.122 ^{a,2}	9.82 ± 0.89 ^{f,1}	8.56 ± 0.32 ^{b,1}	1.15:1 ^{g,12}	77.15 ± 0.13 ^{e,2}
GR-D	0.171 ± 0.006 ^{a,3}	13.155 ± 0.069 ^{a,1}	10.74 ± 0.04 ^{f,2}	7.58 ± 0.34 ^{b,2}	1.42:1 ^{g,1}	77.79 ± 0 ^{e,1}
GR-M	0.245 ± 0.001 ^{a,1}	13.546 ± 0.229 ^{a,1}	7.19 ± 0.38 ^{f,1}	8.35 ± 0.03 ^{b,12}	0.86:1 ^{g,2}	77.97 ± 0 ^{e,1}
FM-S	0.056 ± 0.002 ^{f,1}	5.431 ± 0.074 ^{d,1}	20.43 ± 0.4 ^{a,1}	7.8 ± 0.04 ^{c,2}	2.62:1 ^{c,2}	72 ± 0.26 ^{f,2}
FM-D	0.043 ± 0.000 ^{f,2}	4.199 ± 0.063 ^{d,3}	17.21 ± 0.27 ^{a,2}	5.46 ± 0.03 ^{c,3}	3.15:1 ^{c,1}	76.79 ± 0.13 ^{f,1}
FM-M	0.023 ± 0.000 ^{f,3}	4.995 ± 0.190 ^{d,2}	17.85 ± 0.31 ^{a,2}	8.38 ± 0.15 ^{c,1}	2.13:1 ^{c,3}	72.45 ± 0.38 ^{f,2}
FA-S	0.091 ± 0.003 ^{d,1}	6.475 ± 0.072 ^{c,1}	13.4 ± 0.07 ^{c,2}	3.03 ± 0.1 ^{e,3}	4.42:1 ^{b,1}	72.45 ± 1.15 ^{g,12}
FA-D	0.041 ± 0.001 ^{d,3}	5.692 ± 0.012 ^{c,2}	15.44 ± 0.38 ^{c,1}	5.87 ± 0.16 ^{e,1}	2.63:1 ^{b,3}	71 ± 0.38 ^{g,2}
FA-M	0.064 ± 0.002 ^{d,2}	5.457 ± 0.069 ^{c,3}	13.62 ± 0.77 ^{c,2}	3.79 ± 0.04 ^{e,2}	3.59:1 ^{b,2}	74.44 ± 0.38 ^{g,1}

BD: Bombay duck (*Harpadon nehereus*); RF: ribbon fish (*Trichiurus iepturus*); WS: white sardine (*Escualosa thoracata*); FB: freshwater barb (*Puntius spp.*); GR: Ganges River sprat (*Corica soborna*); FM: fermented barb (*Puntius spp.*); FA: fermented anchovies (*Setipnna spp.*); C: Cox's Bazar; D: Dhaka; M: Mymensingh; S: Sylhet. N/A: data not available due to the sample size. Different letters (a, b, and c) represent significant differences between fish types via two-way ANOVA (p<0.05). Different numbers (1, 2, and 3) represent significant differences within the same fish type and different locations via one-way ANOVA (p<0.05).

fish. Thus, it is believed that the DFs in present study may have the potential to achieve a higher digestibility when cooked.

3.3.6 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 the lowest total free amino acid (TFAA) content, with RF-M containing 187.69 ± 5.23 (mg/100 g protein), when compared to FA and FM (5423.83 ± 60.64 - 9310.53 ± 206.15) and FM (4486.72 ± 175.77 - 5423.83 ± 60.64 mg/100 g protein; Table 7). The significantly high TFAA content in fermented DFs is believed to be related to the fermentation process, in which 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 TFAA of the product reached a 1.4-fold increment after the ripening stage, which is consistent with what we found in the current study: DFs (FB and FM), which are from the same species (*Puntius spp*.) but the TFAA content in the fermented FM were 1.2- to 2.8-fold higher than the unfermented FB. In current study, the most abundant FAA in DFs was glutamic (20.94 ± 0.90 - 1626.38 ± 83.77 mg/100 g protein), followed by alanine (10.38 ± 0.53 - 1267.28 ± 51.95 mg/100 g protein), leucine (16.21 ± 0.06 - 1197.67 ± 13.77 mg/100 g protein), and lysine (28.93 ± 0.52 - 858.04 ± 68.87 mg/100 g protein). The presence of free Glu and Ala can increase consumers' acceptance of DFs because of their 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; Zhao et al., 2016). The presence of Lys and other FAA will also contribute to the Maillard reaction, during which the taste-favorable Maillard reaction and browning compounds

are formed, and consequently enhance acceptance of the DFs (Shah et al., 2009). Apart from the above-mentioned, biogenic amines, a class of naturally occurring low molecular weight compounds usually with a strong odor, are also easily generated from the FAA-rich environments (such as DFs) and are important factors affecting the sensory quality of DFs (Rabie et al., 2009).

Table 7. Free amino acid content in DFs mg/100g protein

	BD-C	BD-D	BD-M	RF-C	RF-D	RF-M
His	20.56 ± 5.53 ^{ef,1}	8.04 ± 0.38 ^{ef,2}	5.24 ± 2.16 ^{ef,2}	10.33 ± 2.04 ^{f,1}	6.36 ± 0.35 ^{f,1}	7.99 ± 2.21 ^{f,1}
Ser	78.34 ± 8.14 ^{d,1}	72.37 ± 8.01 ^{d,1}	63.56 ± 4.19 ^{d,1}	60.47 ± 0.02 ^{f,1}	29.27 ± 0.40 ^{f,2}	5.26 ± 0.24 ^{f,3}
Arg	154.71 ± 18.81 ^{d,1}	21.74 ± 1.28 ^{d,3}	81.06 ± 7.65 ^{d,2}	58.73 ± 1.33 ^{f,1}	15.77 ± 0.96 ^{f,2}	18.88 ± 0.88 ^{f,2}
Gly	179.93 ± 24.42 ^{c,1}	123.01 ± 8.92 ^{c,2}	79.48 ± 6.13 ^{c,2}	72.45 ± 0.07 ^{e,2}	102.70 ± 3.70 ^{e,1}	1.53 ± 0.03 ^{e,3}
Asx	160.52 ± 12.96 ^{b,2}	323.03 ± 37.85 ^{b,1}	142.68 ± 4.68 ^{b,2}	135.85 ± 1.63 ^{d,1}	90.19 ± 6.68 ^{d,2}	24.36 ± 0.05 ^{d,3}
Glx	671.95 ± 57.50 ^{c,2}	486.66 ± 67.13 ^{c,1}	354.00 ± 15.86 ^{c,2}	284.29 ± 3.92 ^{f,1}	300.13 ± 18.21 ^{f,1}	20.94 ± 0.90 ^{f,2}
Thr	146.39 ± 7.77 ^{e,1}	58.82 ± 6.30 ^{e,3}	92.59 ± 5.65 ^{e,2}	74.29 ± 0.25 ^{g,1}	46.35 ± 1.19 ^{g,2}	5.59 ± 0.27 ^{g,3}
Ala	439.86 ± 25.88 ^{d,1}	438.47 ± 65.70 ^{d,1}	267.84 ± 19.18 ^{d,2}	188.02 ± 5.39 ^{f,2}	258.88 ± 15.50 ^{f,1}	10.38 ± 0.53 ^{f,3}
Pro	155.06 ± 17.10 ^{c,1}	94.10 ± 8.31 ^{c,2}	101.42 ± 6.79 ^{c,2}	45.06 ± 0.95 ^{e,2}	60.56 ± 0.89 ^{e,1}	5.97 ± 0.34 ^{e,3}
Cys	35.59 ± 2.52 ^{b,1}	0.00 ± 0.00 ^{b,2}	1.97 ± 0.14 ^{b,2}	5.81 ± 0.32 ^{d,1}	0.00 ± 0.00 ^{d,2}	0.00 ± 0.00 ^{d,2}
Lys	488.92 ± 25.61 ^{c,1}	121.49 ± 20.48 ^{c,2}	166.05 ± 7.31 ^{c,2}	78.18 ± 1.22 ^{e,1}	83.14 ± 9.43 ^{e,1}	28.93 ± 0.52 ^{e,2}
Tyr	509.03 ± 54.30 ^{a,1}	510.18 ± 75.00 ^{a,1}	234.61 ± 14.05 ^{a,2}	96.68 ± 1.91 ^{f,2}	129.40 ± 2.73 ^{f,1}	5.95 ± 0.62 ^{f,3}
Met	149.10 ± 17.68 ^{b,1}	47.67 ± 2.95 ^{b,2}	51.04 ± 3.26 ^{b,2}	19.43 ± 0.85 ^{d,2}	32.06 ± 1.31 ^{d,1}	5.38 ± 0.25 ^{d,3}
Val	344.67 ± 35.11 ^{bc,1}	267.89 ± 34.07 ^{bc,1}	205.68 ± 12.69 ^{bc,1}	112.24 ± 0.09 ^{f,2}	128.42 ± 1.76 ^{f,1}	10.18 ± 1.14 ^{f,3}
Ile	296.38 ± 31.11 ^{c,1}	172.44 ± 21.30 ^{c,2}	156.52 ± 10.63 ^{c,2}	82.23 ± 1.11 ^{f,2}	87.13 ± 1.00 ^{f,1}	5.16 ± 0.02 ^{f,3}
Leu	600.53 ± 59.23 ^{c,1}	276.63 ± 28.32 ^{c,2}	267.32 ± 15.96 ^{c,2}	114.94 ± 0.78 ^{e,2}	136.30 ± 2.18 ^{e,1}	16.21 ± 0.06 ^{e,3}
Phe	273.32 ± 37.47 ^{c,1}	89.25 ± 4.87 ^{c,2}	132.78 ± 10.88 ^{c,2}	54.81 ± 1.24 ^{f,1}	56.72 ± 4.68 ^{f,1}	7.09 ± 0.36 ^{f,2}
Trp	150.30 ± 5.38 ^{c,1}	100.47 ± 1.27 ^{c,2}	44.37 ± 0.37 ^{c,3}	46.92 ± 1.63 ^{f,2}	59.59 ± 1.26 ^{f,1}	7.87 ± 0.03 ^{f,3}
Total	4855.16 ± 446.53 ^{c,1}	3212.25 ± 392.14 ^{c,2}	2448.21 ± 146.85 ^{c,2}	1540.74 ± 15.54 ^{f,2}	1622.97 ± 43.91 ^{f,1}	187.69 ± 5.23 ^{f,3}

Table 7. - *contd.*

	WS-C	WS-D	WS-M	FB-S	FB-D	FB-M
His	138.28 ± 12.37 b,2	191.72 ± 7.64 b,1	120.56 ± 12.30 b,2	131.99 ± 9.42 c,1	104.65 ± 8.90 c,2	133.10 ± 1.33 c,1
Ser	124.28 ± 0.04 b,1	79.15 ± 1.00 b,3	99.85 ± 3.51 b,2	131.43 ± 2.38 c,1	81.82 ± 6.10 c,2	72.56 ± 1.87 c,2
Arg	194.19 ± 5.83 b,12	178.52 ± 3.18 b,2	209.46 ± 10.09 b,1	135.67 ± 0.42 c,1	113.49 ± 10.63 c,2	104.33 ± 2.48 c,2
Gly	111.06 ± 5.37 cd,2	113.14 ± 2.76 cd,2	142.55 ± 7.00 cd,1	132.50 ± 3.40 cd,1	94.89 ± 9.66 cd,2	136.03 ± 2.22 cd,1
Asx	169.15 ± 7.49 c,1	127.74 ± 5.08 c,2	165.61 ± 3.64 c,1	222.11 ± 7.87 c,1	131.85 ± 4.25 c,2	97.60 ± 4.00 c,3
Glx	420.11 ± 22.48 d,1	362.12 ± 16.99 d,2	463.15 ± 12.15 d,1	543.85 ± 8.37 de,1	311.59 ± 14.07 de,2	184.75 ± 9.08 de,3
Thr	176.58 ± 2.74 a,1	124.93 ± 1.54 a,3	160.50 ± 6.22 a,2	172.22 ± 3.93 c,1	105.06 ± 7.67 c,2	71.39 ± 2.10 c,3
Ala	432.05 ± 22.92 c,12	395.22 ± 17.28 c,1	470.21 ± 7.35 c,1	379.60 ± 14.44 e,1	239.01 ± 10.70 e,2	157.75 ± 6.47 e,3
Pro	145.55 ± 1.92 b,2	123.44 ± 1.75 b,3	163.48 ± 3.59 b,1	117.03 ± 1.59 d,1	73.24 ± 5.38 d,2	62.95 ± 1.69 d,2
Cys	20.84 ± 2.21 b,1	7.47 ± 2.06 b,2	7.83 ± 0.41 b,2	4.16 ± 1.62 d,1	2.18 ± 1.90 d,1	0.00 ± 0.00 d,1
Lys	236.01 ± 19.70 d,1	181.90 ± 19.29 d,2	201.28 ± 2.63 d,12	368.08 ± 30.34 cd,1	192.41 ± 5.28 cd,2	171.50 ± 11.16 cd,2
Tyr	185.19 ± 1.53 c,2	178.29 ± 7.13 c,2	246.72 ± 10.56 c,1	163.73 ± 11.04 de,1	108.27 ± 5.85 de,2	54.59 ± 1.15 de,3
Met	97.37 ± 0.96 c,1	50.97 ± 0.89 c,3	62.98 ± 3.16 c,2	29.29 ± 0.54 d,1	14.76 ± 0.84 d,2	5.95 ± 0.23 d,3
Val	260.88 ± 4.72 c,1	200.09 ± 4.38 c,2	266.68 ± 6.64 c,1	269.48 ± 7.20 e,1	152.28 ± 12.55 e,2	92.28 ± 3.64 e,3
Ile	198.31 ± 0.21 d,1	144.90 ± 1.60 d,2	193.00 ± 3.39 d,1	216.53 ± 4.88 e,1	115.01 ± 7.23 e,2	68.33 ± 3.08 e,3
Leu	426.67 ± 4.17 c,1	293.20 ± 5.29 c,3	369.97 ± 13.08 c,2	400.62 ± 9.00 d,1	206.35 ± 16.52 d,2	126.97 ± 4.16 d,3
Phe	177.05 ± 11.26 cd,1	115.14 ± 3.88 cd,3	146.72 ± 7.86 cd,2	168.07 ± 6.21 e,1	88.06 ± 9.59 e,2	51.11 ± 0.57 e,3
Trp	204.78 ± 4.85 b,1	99.40 ± 2.76 b,3	117.29 ± 1.56 b,2	90.18 ± 2.56 e,1	51.97 ± 0.90 e,2	23.72 ± 0.32 e,3
Total	3718.35 ± 45.56 c,1	2967.33 ± 48.02 c,2	3607.84 ± 109.05 c,1	3676.55 ± 54.79 e,1	2186.90 ± 134.23 e,2	1614.91 ± 52.15 e,3

Table 7. - *contd.*

	GR-S	GR-D	GR-M	FM-S	FM-D	FM-M	FA-S	FA-D	FA-M
His	456.93 ± 36.41 ^{a,1}	345.27 ± 16.96 ^{a,2}	537.36 ± 34.98 ^{a,1}	72.70 ± 3.49 ^{d,2}	97.26 ± 9.37 ^{d,1}	49.78 ± 5.52 ^{d,3}	18.47 ± 6.51 ^{e,2}	43.53 ± 2.91 ^{e,1}	16.29 ± 3.17 ^{e,2}
Ser	111.65 ± 3.59 ^{a,2}	151.51 ± 7.66 ^{a,1}	128.13 ± 11.79 ^{a,12}	24.23 ± 0.28 ^{g,2}	41.65 ± 1.33 ^{g,1}	6.63 ± 0.09 ^{g,3}	30.01 ± 5.71 ^{e,2}	101.23 ± 1.48 ^{e,1}	32.02 ± 0.25 ^{e,2}
Arg	356.81 ± 7.55 ^{a,1}	349.18 ± 25.20 ^{a,1}	269.82 ± 22.49 ^{a,2}	52.32 ± 1.85 ^{e,2}	85.12 ± 6.19 ^{e,1}	55.68 ± 1.98 ^{e,2}	50.63 ± 7.36 ^{f,1}	30.51 ± 0.49 ^{f,2}	29.60 ± 2.79 ^{f,2}
Gly	99.67 ± 6.51 ^{d,1}	118.07 ± 4.21 ^{d,1}	101.64 ± 8.08 ^{d,1}	162.31 ± 8.38 ^{b,2}	140.38 ± 11.97 ^{b,2}	225.46 ± 10.58 ^{b,1}	278.54 ± 35.20 ^{a,2}	390.22 ± 9.67 ^{a,1}	295.20 ± 26.65 ^{a,2}
Asx	103.00 ± 1.67 ^{d,1}	132.32 ± 10.44 ^{d,1}	102.69 ± 13.02 ^{d,1}	311.19 ± 16.63 ^{b,1}	272.99 ± 2.66 ^{b,2}	105.57 ± 6.57 ^{b,3}	494.76 ± 94.12 ^{a,1}	509.26 ± 23.24 ^{a,1}	419.07 ± 25.05 ^{a,1}
Glx	301.41 ± 12.67 ^{e,12}	366.65 ± 28.37 ^{e,1}	240.48 ± 28.98 ^{e,2}	667.21 ± 36.96 ^{b,2}	606.35 ± 8.71 ^{b,2}	835.88 ± 56.98 ^{b,1}	1348.01 ± 241.81 ^{a,12}	1626.38 ± 83.77 ^{a,1}	1112.67 ± 45.44 ^{a,2}
Thr	130.00 ± 1.86 ^{b,12}	144.07 ± 7.83 ^{b,1}	110.17 ± 9.73 ^{b,2}	55.51 ± 0.87 ^{f,2}	78.80 ± 3.54 ^{f,1}	15.43 ± 0.48 ^{f,3}	34.74 ± 5.36 ^{d,3}	183.08 ± 3.88 ^{d,1}	104.63 ± 0.03 ^{d,2}
Ala	337.23 ± 3.97 ^{d,1}	383.76 ± 25.54 ^{d,1}	310.07 ± 33.64 ^{d,1}	600.75 ± 20.29 ^{b,2}	380.93 ± 0.93 ^{b,3}	906.03 ± 51.56 ^{b,1}	823.70 ± 148.12 ^{a,2}	1267.28 ± 51.95 ^{a,1}	739.23 ± 22.81 ^{a,2}
Pro	151.01 ± 5.70 ^{b,12}	156.49 ± 8.57 ^{b,1}	129.18 ± 10.29 ^{b,2}	126.70 ± 0.40 ^{c,2}	97.63 ± 2.83 ^{c,3}	143.70 ± 2.33 ^{c,1}	255.76 ± 34.04 ^{a,1}	257.35 ± 3.49 ^{a,1}	166.04 ± 4.86 ^{a,2}
Cys	15.39 ± 1.29 ^{b,1}	10.37 ± 1.30 ^{b,2}	3.82 ± 0.92 ^{b,3}	7.22 ± 3.01 ^{c,1}	2.78 ± 3.93 ^{c,1}	5.99 ± 0.26 ^{c,1}	20.11 ± 7.44 ^{a,12}	31.62 ± 0.69 ^{a,1}	9.71 ± 2.74 ^{a,2}
Lys	258.95 ± 15.92 ^{c,1}	299.14 ± 31.03 ^{c,1}	230.97 ± 27.15 ^{c,1}	523.39 ± 35.83 ^{b,1}	482.34 ± 23.50 ^{b,1}	624.21 ± 68.67 ^{b,1}	614.82 ± 129.05 ^{a,1}	858.04 ± 68.87 ^{a,1}	596.10 ± 50.70 ^{a,1}
Tyr	164.14 ± 2.80 ^{d,1}	133.84 ± 1.88 ^{d,2}	94.31 ± 4.53 ^{d,3}	148.11 ± 1.66 ^{ef,1}	96.02 ± 14.98 ^{ef,2}	59.79 ± 8.34 ^{ef,3}	271.91 ± 28.77 ^{b,2}	397.70 ± 27.83 ^{b,1}	271.79 ± 8.60 ^{b,2}
Met	73.53 ± 2.69 ^{c,1}	72.31 ± 2.77 ^{c,1}	41.12 ± 2.01 ^{c,2}	69.42 ± 0.41 ^{c,2}	36.93 ± 2.15 ^{c,3}	91.39 ± 0.56 ^{c,1}	295.69 ± 40.65 ^{a,1}	261.83 ± 1.08 ^{a,1}	140.88 ± 5.18 ^{a,2}
Val	188.12 ± 3.43 ^{e,1}	203.08 ± 11.09 ^{e,1}	142.98 ± 11.06 ^{e,2}	341.21 ± 3.76 ^{b,1}	222.46 ± 6.62 ^{b,2}	340.27 ± 10.49 ^{b,1}	649.08 ± 107.17 ^{a,1}	693.06 ± 10.67 ^{a,1}	379.92 ± 0.77 ^{a,2}
Ile	133.61 ± 0.14 ^{e,1}	142.94 ± 10.72 ^{e,1}	101.62 ± 8.64 ^{e,2}	310.18 ± 3.71 ^{b,1}	174.03 ± 6.62 ^{b,3}	272.80 ± 5.37 ^{b,2}	620.74 ± 103.22 ^{a,1}	633.92 ± 5.85 ^{a,1}	245.25 ± 4.17 ^{a,2}
Leu	308.50 ± 4.45 ^{d,1}	317.74 ± 16.78 ^{d,1}	247.77 ± 20.15 ^{d,2}	564.50 ± 1.18 ^{b,1}	378.64 ± 8.20 ^{b,3}	474.85 ± 11.47 ^{b,2}	1181.06 ± 187.31 ^{a,1}	1197.67 ± 13.77 ^{a,1}	517.16 ± 6.16 ^{a,2}
Phe	157.06 ± 8.42 ^{d,1}	149.53 ± 6.62 ^{d,1}	122.28 ± 9.95 ^{d,2}	193.12 ± 9.50 ^{b,12}	176.93 ± 18.46 ^{b,2}	229.49 ± 10.91 ^{b,1}	401.90 ± 47.35 ^{a,2}	545.11 ± 12.46 ^{a,1}	242.13 ± 25.33 ^{a,3}
Trp	124.49 ± 2.52 ^{c,1}	113.22 ± 3.88 ^{c,2}	64.43 ± 0.90 ^{c,3}	150.50 ± 3.88 ^{d,1}	43.56 ± 0.40 ^{d,2}	43.80 ± 0.61 ^{d,2}	220.89 ± 6.19 ^{a,2}	282.76 ± 5.67 ^{a,1}	106.15 ± 4.52 ^{a,3}
Total	3471.49 ± 50.27 ^{d,1}	3589.48 ± 213.08 ^{d,1}	2978.83 ± 254.65 ^{d,2}	4380.58 ± 90.60 ^{b,1}	3414.79 ± 77.53 ^{b,2}	4486.72 ± 175.77 ^{b,1}	7610.82 ± 1223.00 ^{a,12}	9310.53 ± 206.15 ^{a,1}	5423.83 ± 60.64 ^{a,2}

BD: Bombay duck (Harpadon nehereus); RF: ribbon fish (Trichiurus iepturus); WS: white sardine (Escualosa thoracata); FB: freshwater barb (Puntius spp.); GR: Ganges River sprat (Corica soborna); FM: fermented barb (Puntius spp.); FA: fermented anchovies (Setipnna spp.); C: Cox's Bazar; D: Dhaka; M: Mymensingh; S: Sylhet. Different letters (a, b, and c) represent significant differences between fish types via two-way ANOVA ($p<0.05$). Different numbers (1, 2, and 3) represent significant differences within the same fish type and different locations via one-way ANOVA ($p<0.05$); His: histidine; Arg: arginine; Thr : threonine; Val: valine; Met: methionine; Ile: isoleucine; Leu: leucine; Phe: phenylalanine; Trp: tryptophan; Lys: lysine; Ala: alanine; Asx- Aspartic acid +asparagine; Cys: cysteine; Glx- Glutamic acid +glutamine; Gly: glycine; Pro: proline; Ser: serine; Tyr; tyrosine.

3.3.7 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 6 lists the yields of sarcoplasmic and myofibrillar protein of each DF and their ratio. Overall, the yields of sarcoplasmic protein were in the range of 3.83 ± 0.01 to 20.43 ± 0.4 %, which are higher than the 2.1 ± 0.09 - 11.38 ± 0.12 % for the myofibrillar protein. The high ratio (0.86:1 to 6.81:1) of sarcoplasmic to myofibrillar proteins indicate that the myofibrillar proteins were degraded during processing due to the activity of endogenous proteolytic enzymes namely, cathepsins, serine proteases, collagenases and calpains (Yang et al., 2015). In addition, Niu et al. (2019) suggested that microbes involved in the fermentation process also play a crucial role in decomposition and degradation of muscle protein. Degradation of both myofibrillar protein and sarcoplasmic protein will not only affect their functional properties but also result in increases in the contents of short-chain peptides and free amino acids (Visessanguan et al., 2004), which may contribute to the taste, aroma, and nutritional quality (increased protein digestibility) of DFs.

3.3.8 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 as shown in Table 7. In the present study, SFA are predominant, accounting for about 41.39 ± 0.11 - 60.91 ± 0.47% of the total fatty acids. Among all eleven detected SFA, the content of palmitic (C16:0) was highest, ranging from 25.54 ± 0.07 to 38.18 ± 0.05 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, with values in the ranges of 2.99 ± 0.02 to 12.44 ± 0.084 and 5.55 ± 0.01 to 36.16 ± 0.10, respectively. When it comes to the PUFA composition, different DFs showed varying characteristics. In terms of n-6 fatty acids, arachidonic (C20:4) whose content ranged 1.68 ± 0.011 to 4.91 ± 0.04 g/100 g of total fatty acids is one of the most abundant n-6 fatty acids detected in the DFs. Linoleic acid (C18:2), whose content is in the 0.73 ± 0.01 - 9.91 ± 0.052 g/100 g of total fatty acids range, was also enriched in DFs, and even became the most abundant n-6 fatty acids in FB, GR and FM. As for n-3 fatty acids, docosahexaenoic (DHA, C22:6) and eicosapentaenoic acid (EPA, C20:5) were the most abundant in BD, RF, WS, GR, FA with a range of 7.20 ± 0.12 - 19.87 ± 0.07, and 1.81 ± 0.04 - 7.47 ± 0.01 g/100 g of total fatty acids, respectively. In contrast, α-linolenic acid (C18:3), whose content ranged from 2.50 ± 0.02 to 6.00 ± 0.01 g/100 g of total fatty acids, was the predominant n-3 fatty acids in FB, FM and GR. 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 in a previous study on whole *Puntius spp*. (FB) powder (Bhowmik et al., 2022). The same study also reported the fatty acid profile of whole *Corica soborna* (GR) powder, with 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 consistent with 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 reducing 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 (7.92 ± 0.04 - 9.03 ± 0.04) is significantly higher than 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 in the western diet 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, the PUFA/SFA ratios of WS (0.67 ± 0.00

- 0.73 ±0.01) and GR (0.78 ± 0.01 - 0.88 ± 0.00) are higher than 0.4, and significantly higher than the ratios for other DFs. In the sense of reducing obesity risk, addition of WS and GR to the diet could be a useful approach.

Table 8. Fatty acid composition (g/100 g of total fatty acids) of dried fishes

Fatty acid	BD-C	BD-D	BD-M
Caprylic acid (C8:0)	n.d.	n.d.	n.d.
Capric acid (C10:0)	0.009 ± 0.000	0.008 ± 0.002	0.009 ± 0.000
Lauric acid (C12:0)	0.483 ± 0.035	0.236 ± 0.021	0.306 ± 0.000
Myristic acid (C14:0)	4.817 ± 0.207	6.015 ± 0.547	7.078 ± 0.049
Pentadecylic acid (C15:0)	0.653 ± 0.023	0.604 ± 0.062	0.506 ± 0.001
Palmitic acid (C16:0)	34.235 ± 0.810	32.758 ± 2.862	38.178 ± 0.054
Margaric acid (C17:0)	0.966 ± 0.025	0.719 ± 0.066	0.610 ± 0.000
Stearic acid (C18:0)	8.873 ± 0.016	6.178 ± 0.570	6.231 ± 0.038
Arachidic acid (C20:0)	0.482 ± 0.039	0.408 ± 0.059	0.327 ± 0.002
Behenic acid (C22:0)	0.772 ± 0.066	0.507 ± 0.103	0.507 ± 0.011
Lignoceric acid (C24:0)	0.756 ± 0.032	0.459 ± 0.106	0.418 ± 0.006
∑SFA	52.044 ± 1.252 ^{b,1}	47.890 ± 3.862 ^{b,1}	54.169 ± 0.049 ^{b,1}
Myristoleic acid (C14:1)	0.054 ± 0.006	0.107 ± 0.009	0.076 ± 0.001
Palmitoleic acid (C16:1)	8.931 ± 0.223	9.888 ± 0.571	12.437 ± 0.084
Palmitolaidic acid (C16:1t)	0.302 ± 0.001	0.282 ± 0.016	0.238 ± 0.003
Oleic acid (C18:1)	11.467 ± 0.088	14.718 ± 0.216	12.483 ± 0.117
Vaccenic acid (C18:1n7c)	3.128 ± 0.033	2.840 ± 0.146	2.715 ± 0.009
Eicosenoic acid (C20:1)	0.524 ± 0.013	0.781 ± 0.102	0.536 ± 0.002
Erucic acid (C22:1)	0.055 ± 0.004	0.102 ± 0.016	0.037 ± 0.004
Nervonic acid (C24:1)	0.688 ± 0.078	0.441 ± 0.052	0.314 ± 0.004
∑MUFA	25.148 ± 0.422 ^{c,2}	19.439 ± 16.843 ^{c,1}	28.835 ± 0.203 ^{c,1}
Linoleic acid (n-6, C18:2)	0.842 ± 0.014	0.728 ± 0.007	0.726 ± 0.008
γ -Linolenic acid (n-6, C18:3n6)	0.333 ± 0.001	0.195 ± 0.004	0.276 ± 0.003
α-Linolenic acid (n-3, C18:3n3)	0.577 ± 0.001	0.295 ± 0.011	0.402 ± 0.003
Eicosadienoic acid (n-6, C20:2)	0.221 ± 0.008	0.185 ± 0.039	0.154 ± 0.003
Eicosatrienoic acid (n-6, C20:3n6)	0.135 ± 0.007	0.144 ± 0.025	0.119 ± 0.007
Arachidonic acid (n-6, C20:4)	2.340 ± 0.148	3.176 ± 0.471	1.954 ± 0.024
Ecosatrienoic acid (n-3, C20:3n3)	0.186 ± 0.004	0.147 ± 0.088	0.099 ± 0.000
Eicosapentaenoic acid, EPA (n-3, C20:5)	5.176 ± 0.326	4.979 ± 1.165	4.508 ± 0.071
Docosadienoic acid (n-6, C22:2)	0.015 ± 0.001	0.031 ± 0.001	0.006 ± 0.000
Adrenic acid (n-6, C22:4)	0.249 ± 0.025	0.379 ± 0.055	0.196 ± 0.004
Docosapentaenoic acid (n-6, C22:5n6)	0.749 ± 0.081	0.846 ± 0.137	0.521 ± 0.008
Docosapentaenoic acid, DPA (n-3, C22:5n3)	0.951 ± 0.076	1.142 ± 0.202	0.838 ± 0.021
Docosahexanoic acid, DHA (n-3, C22:6n3)	11.034 ± 1.036	10.707 ± 2.478	7.201 ± 0.118
∑PUFA	22.870 ± 1.674 ^{d,1}	22.593 ± 4.635 ^{d,1}	16.998 ± 0.252 ^{d,1}
UFA	47.955 ± 1.252 ^{de,1}	52.111 ± 3.866 ^{de,1}	45.832 ± 0.049 ^{de,1}
∑n-3	17.923 ± 1.433 ^{d,1}	17.270 ± 3.921 ^{d,1}	13.047 ± 0.212 ^{d,1}
∑n-6	2.544 ± 0.093 ^{e,1}	2.507 ± 0.243 ^{e,1}	1.997 ± 0.016 ^{e,2}
n-3/n-6	7.040 ± 0.305 ^{b,1}	6.845 ± 0.900 ^{b,1}	6.535 ± 0.053 ^{b,1}
PUFA/SFA	0.439 ± 0.043 ^{c,1}	0.485 ± 0.136 ^{c,1}	0.314 ± 0.005 ^{c,1}

Table 8. - *contd.*

Fatty acid	RF-C	RF-D	RF-M
Caprylic acid (C8:0)	n.d.	n.d.	n.d.
Capric acid (C10:0)	0.004 ± 0.001	0.004 ± 0.000	0.002 ± 0.000
Lauric acid (C12:0)	0.070 ± 0.001	0.064 ± 0.001	0.049 ± 0.001
Myristic acid (C14:0)	4.804 ± 0.138	3.343 ± 0.007	3.265 ± 0.023
Pentadecylic acid (C15:0)	0.851 ± 0.005	0.998 ± 0.001	0.873 ± 0.008
Palmitic acid (C16:0)	32.239 ± 0.334	26.157 ± 0.093	36.970 ± 0.433
Margaric acid (C17:0)	1.139 ± 0.009	1.397 ± 0.000	1.490 ± 0.013
Stearic acid (C18:0)	11.505 ± 0.095	13.761 ± 0.079	16.005 ± 0.163
Arachidic acid (C20:0)	0.419 ± 0.004	0.539 ± 0.008	0.666 ± 0.006
Behenic acid (C22:0)	0.323 ± 0.001	0.440 ± 0.025	0.581 ± 0.002
Lignoceric acid (C24:0)	0.637 ± 0.014	1.046 ± 0.042	1.009 ± 0.001
∑SFA	51.989 ± 0.359 ^{a,2}	47.747 ± 0.239 ^{a,3}	60.906 ± 0.469 ^{a,1}
Myristoleic acid (C14:1)	0.037 ± 0.005	0.015 ± 0.003	0.007 ± 0.001
Palmitoleic acid (C16:1)	6.986 ± 0.173	3.659 ± 0.018	2.987 ± 0.021
Palmitolaidic acid (C16:1t)	0.303 ± 0.006	0.255 ± 0.004	0.162 ± 0.005
Oleic acid (C18:1)	16.480 ± 0.127	12.722 ± 0.044	10.702 ± 0.087
Vaccenic acid (C18:1n7c)	3.357 ± 0.011	3.050 ± 0.010	2.514 ± 0.016
Eicosenoic acid (C20:1)	0.458 ± 0.009	0.504 ± 0.004	0.354 ± 0.003
Erucic acid (C22:1)	0.057 ± 0.004	0.073 ± 0.007	0.050 ± 0.001
Nervonic acid (C24:1)	0.920 ± 0.014	1.635 ± 0.083	1.190 ± 0.006
∑MUFA	28.597 ± 0.295 ^{e,1}	21.912 ± 0.021 ^{e,2}	17.964 ± 0.129 ^{e,3}
Linoleic acid (n-6, C18:2)	0.951 ± 0.009	1.118 ± 0.005	1.260 ± 0.009
γ -Linolenic acid (n-6, C18:3n6)	0.348 ± 0.007	0.157 ± 0.002	0.134 ± 0.007
α-Linolenic acid (n-3, C18:3n3)	0.331 ± 0.004	0.226 ± 0.003	0.174 ± 0.004
Eicosadienoic acid (n-6, C20:2)	0.162 ± 0.010	0.232 ± 0.000	0.171 ± 0.001
Eicosatrienoic acid (n-6, C20:3n6)	0.239 ± 0.001	0.236 ± 0.001	0.171 ± 0.001
Arachidonic acid (n-6, C20:4)	2.072 ± 0.083	4.124 ± 0.048	1.990 ± 0.043
Ecosatrienoic acid (n-3, C20:3n3)	0.082 ± 0.005	0.085 ± 0.001	0.076 ± 0.008
Eicosapentaenoic acid, EPA (n-3, C20:5)	2.537 ± 0.042	2.636 ± 0.037	1.809 ± 0.040
Docosadienoic acid (n-6, C22:2)	0.014 ± 0.005	0.025 ± 0.001	0.018 ± 0.000
Adrenic acid (n-6, C22:4)	0.433 ± 0.000	0.847 ± 0.010	0.461 ±0.011
Docosapentaenoic acid (n-6, C22:5n6)	0.904 ± 0.034	1.986 ± 0.015	1.172 ± 0.029
Docosapentaenoic acid, DPA (n-3, C22:5n3)	1.473 ±0.011	1.612 ± 0.005	1.345 ± 0.018
Docosahexanoic acid, DHA (n-3, C22:6n3)	9.873 ± 0.489	17.002 ± 0.155	12.354 ± 0.347
∑PUFA	19.416 ± 0.665 ^{c,3}	30.342 ± 0.260 ^{c,1}	21.132 ± 0.518 ^{c,2}
UFA	48.012 ± 0.361 ^{e,2}	52.254 ± 0.239 ^{e,1}	39.096 ± 0.647 ^{e,3}
∑n-3	14.295 ± 0.521 ^{c,3}	21.619 ± 0.199 ^{c,1}	15.757 ± 0.418 ^{c,2}
∑n-6	3.050 ± 0.052 ^{c,3}	4.599 ± 0.013 ^{c,1}	3.386 ± 0.057 ^{c,2}
n-3/n-6	4.687 ± 0.092 ^{c,1}	4.701 ± 0.030 ^{c,1}	4.653 ± 0.046 ^{c,1}
PUFA/SFA	0.374 ± 0.015 ^{c,2}	0.635 ± 0.009 ^{c,1}	0.347 ± 0.012 ^{c,2}

Table 8. - *contd.*

Fatty acid	WS-C	WS-D	WS-M
Caprylic acid (C8:0)	n.d.	n.d.	n.d.
Capric acid (C10:0)	0.008 ± 0.001	0.008 ± 0.000	0.009 ± 0.001
Lauric acid (C12:0)	0.117 ± 0.000	0.212 ± 0.002	0.239 ± 0.001
Myristic acid (C14:0)	5.738 ± 0.014	4.550 ± 0.014	5.371 ± 0.037
Pentadecylic acid (C15:0)	0963 ± 0.001	1.027 ± 0.004	1.079 ± 0.002
Palmitic acid (C16:0)	30.244 ± 0.036	28.643 ± 0.039	28.692 ± 0.028
Margaric acid (C17:0)	1.244 ± 0.011	1.426 ± 0.000	1.325 ± 0.004
Stearic acid (C18:0)	9.212 ± 0.031	10.864 ± 0.069	9.845 ± 0.005
Arachidic acid (C20:0)	0.419 ± 0.001	0.459 ± 0.005	0.487 ± 0.001
Behenic acid (C22:0)	0.371 ± 0.013	0.549 ± 0.009	0.648 ± 0.001
Lignoceric acid (C24:0)	0.908 ± 0.027	1.296 ± 0.003	1.048 ± 0.059
∑SFA	49.222 ± 0.023 ^{c,1}	49.032 ± 0.100 ^{c,12}	48.740 ± 0.128 ^{c,2}
Myristoleic acid (C14:1)	0.013 ± 0.001	0.009 ± 0.000	0.012 ± 0.000
Palmitoleic acid (C16:1)	6.238 ± 0.033	4.900 ± 0.025	5.939 ± 0.035
Palmitolaidic acid (C16:1t)	0.178 ± 0.008	0.147 ± 0.006	0.177 ± 0.003
Oleic acid (C18:1)	6.360 ± 0.054	5.835 ± 0.032	5.548 ± 0.006
Vaccenic acid (C18:1n7c)	3.770 ± 0.008	3.301 ± 0.025	3.663 ± 0.004
Eicosenoic acid (C20:1)	0.264 ± 0.001	0.229 ± 0.013	0.185 ± 0.008
Erucic acid (C22:1)	0.064 ± 0.000	0.058 ± 0.002	0.028 ± 0.001
Nervonic acid (C24:1)	0.906 ± 0.013	0.840 ± 0.008	0.649 ± 0.006
∑MUFA	17.792 ± 0.084 ^{f,1}	15.317 ± 0.053 ^{f,3}	16.200 ± 0.039 ^{f,2}
Linoleic acid (n-6, C18:2)	1.051 ± 0.021	0.931 ± 0.008	1.103 ± 0.004
γ -Linolenic acid (n-6, C18:3n6)	0.289 ± 0.001	0.282 ± 0.002	0.277 ± 0.003
α-Linolenic acid (n-3, C18:3n3)	0.516 ± 0.006	0.391 ± 0.001	0.796 ± 0.002
Eicosadienoic acid (n-6, C20:2)	0.200 ± 0.005	0.212 ± 0.007	0.143 ± 0.000
Eicosatrienoic acid (n-6, C20:3n6)	0.165 ± 0.003	0.170 ± 0.002	0.177 ± 0.006
Arachidonic acid (n-6, C20:4)	2.787 ± 0.001	4.908 ± 0.037	3.384 ± 0.025
Ecosatrienoic acid (n-3, C20:3n3)	0.084 ± 0.006	0.089 ± 0.004	0.079 ± 0.004
Eicosapentaenoic acid, EPA (n-3, C20:5)	6.195 ± 0.030	6.022 ± 0.004	7.406 ± 0.055
Docosadienoic acid (n-6, C22:2)	0.014 ± 0.008	0.017 ± 0.003	0.011 ± 0.004
Adrenic acid (n-6, C22:4)	0.233 ± 0.007	0.353 ± 0.006	0.243 ± 0.002
Docosapentaenoic acid (n-6, C22:5n6)	1.217 ± 0.001	1.483 ± 0.010	1.207 ± 0.004
Docosapentaenoic acid, DPA (n-3, C22:5n3)	0.975 ± 0.004	0.932 ± 0.011	0.947 ± 0.004
Docosahexanoic acid, DHA (n-3, C22:6n3)	19.264 ± 0.089	19.866 ± 0.072	19.292 ± 0.091
∑PUFA	32.988 ± 0.060 ^{b,3}	35.654 ± 0.149 ^{b,1}	35.063 ± 0.168 ^{b,2}
UFA	50.779 ± 0.024 ^{cd,2}	50.970 ± 0.096 ^{cd,12}	51.262 ± 0.129 ^{cd,1}
∑n-3	27.033 ± 0.104 ^{a,2}	27.299 ± 0.085 ^{a,2}	28.519 ± 0.141 ^{a,1}
∑n-6	3.168 ± 0.042 ^{d,2}	3.447 ± 0.028 ^{d,1}	3.160 ± 0.001 ^{d,2}
n-3/n-6	8.534 ± 0.147 ^{a,2}	7.921 ± 0.039 ^{a,3}	9.025 ± 0.040 ^{a,1}
PUFA/SFA	0.670 ± 0.001 ^{b,2}	0.727 ± 0.005 ^{b,1}	0.719 ± 0.005 ^{b,1}

Table 8. - *contd.*

Fatty acid	FB-S	FB-D	FB-M
Caprylic acid (C8:0)	0.008 ± 0.001	0.004 ± 0.001	0.005 ± 0.001
Capric acid (C10:0)	0.010 ± 0.000	0.014 ± 0.001	0.023 ± 0.001
Lauric acid (C12:0)	0.212 ± 0.003	0.272 ± 0.005	0.262 ± 0.001
Myristic acid (C14:0)	1.972 ± 0.014	2.040 ± 0.001	2.645 ± 0.013
Pentadecylic acid (C15:0)	0.837 ± 0.010	1.066 ± 0.000	1.487 ± 0.001
Palmitic acid (C16:0)	28.392 ± 0.142	25.542 ± 0.074	26.918 ± 0.071
Margaric acid (C17:0)	1.872 ± 0.001	1.778 ± 0.004	2.135 ± 0.019
Stearic acid (C18:0)	10.427 ± 0.023	10.031 ± 0.051	9.208 ± 0.025
Arachidic acid (C20:0)	0.329 ± 0.004	0.342 ± 0.005	0.495 ± 0.028
Behenic acid (C22:0)	0.210 ± 0.001	0.189 ± 0.006	0.246 ± 0.021
Lignoceric acid (C24:0)	0.178 ± 0.019	0.112 ± 0.016	0.170 ± 0.015
∑SFA	44.445 ± 0.161 ^{e,1}	41.387 ± 0.105 ^{e,3}	43.590 ± 0.113 ^{e,2}
Myristoleic acid (C14:1)	0.083 ± 0.001	0.030 ± 0.001	0.066 ± 0.005
Palmitoleic acid (C16:1)	3.379 ± 0.059	3.210 ± 0.034	5.251 ± 0.028
Palmitolaidic acid (C16:1t)	n.d.	n.d.	n.d.
Oleic acid (C18:1)	27.373 ± 0.052	36.163 ± 0.097	27.575 ± 0.094
Vaccenic acid (C18:1n7c)	2.574 ± 0.004	2.156 ± 0.006	3.296 ± 0.004
Eicosenoic acid (C20:1)	0.741 ± 0.031	0.892 ± 0.004	0.928 ± 0.028
Erucic acid (C22:1)	0.024 ± 0.001	0.093 ± 0.000	0.310 ± 0.013
Nervonic acid (C24:1)	0.155 ± 0.012	0.061 ± 0.007	0.163 ± 0.006
∑MUFA	34.327 ± 0.032 ^{b,3}	42.604 ± 0.064 ^{b,1}	37.587 ± 0.110 ^{b,2}
Linoleic acid (n-6, C18:2)	6.826 ± 0.008	7.544 ± 0.054	7.094 ± 0.017
γ -Linolenic acid (n-6, C18:3n6)	0.412 ± 0.016	0.256 ± 0.011	0.303 ± 0.001
α-Linolenic acid (n-3, C18:3n3)	4.458 ± 0.016	2.504 ± 0.021	3.682 ± 0.001
Eicosadienoic acid (n-6, C20:2)	0.483 ± 0.014	0.456 ± 0.006	0.408 ± 0.010
Eicosatrienoic acid (n-6, C20:3n6)	0.587 ± 0.006	0.363 ± 0.002	0.385 ± 0.003
Arachidonic acid (n-6, C20:4)	2.551 ± 0.001	1.792 ± 0.025	2.151 ± 0.057
Ecosatrienoic acid (n-3, C20:3n3)	0.391 ± 0.009	0.171 ± 0.001	0.228 ± 0.011
Eicosapentaenoic acid, EPA (n-3, C20:5)	0.912 ± 0.025	0.620 ± 0.020	1.026 ± 0.037
Docosadienoic acid (n-6, C22:2)	0.014 ± 0.001	0.012 ± 0.001	0.022 ± 0.000
Adrenic acid (n-6, C22:4)	0.366 ± 0.005	0.285 ± 0.003	0.274 ± 0.004
Docosapentaenoic acid (n-6, C22:5n6)	0.549 ± 0.001	0.407 ± 0.007	0.526 ± 0.016
Docosapentaenoic acid, DPA (n-3, C22:5n3)	0.811 ± 0.041	0.401 ± 0.008	0.611 ± 0.028
Docosahexanoic acid, DHA (n-3, C22:6n3)	2.873 ± 0.020	1.203 ± 0.028	2.115 ± 0.072
∑PUFA	21.230 ± 0.128 ^{e,1}	16.012 ± 0.170 ^{e,3}	18.824 ± 0.226 ^{e,2}
UFA	55.556 ± 0.160 ^{ab},	58.616 ± 0.105 ^{ab,1}	56.410 ± 0.116 ^{ab,}
∑n-3	9.444 ± 0.079 ^{e,1}	4.898 ± 0.078 ^{e,3}	7.661 ± 0.146 ^{e,2}
∑n-6	9.235 ± 0.050 ^{b,1}	9.322 ± 0.066 ^{b,1}	9.012 ± 0.023 ^{b,2}
n-3/n-6	1.023 ± 0.003 ^{f,1}	0.525 ± 0.005 ^{f,3}	0.850 ± 0.014 ^{f,2}
PUFA/SFA	0.478 ± 0.005 ^{c,1}	0.387 ± 0.005 ^{c,3}	0.432 ± 0.006 ^{c,2}

Table 8. - *contd.*

Fatty acid	GR-S	GR-D	GR-M
Caprylic acid (C8:0)	n.d.	n.d.	n.d.
Capric acid (C10:0)	0.009 ± 0.001	0.007 ± 0.001	0.018 ± 0.000
Lauric acid (C12:0)	0.181 ± 0.002	0.152 ± 0.001	0.144 ± 0.003
Myristic acid (C14:0)	3.799 ± 0.004	3.223 ± 0.010	3.639 ± 0.162
Pentadecylic acid (C15:0)	1.162 ± 0.003	1.346 ± 0.003	1.127 ± 0.001
Palmitic acid (C16:0)	29.942 ± 0.165	27.648 ± 0.068	27.250 ± 0.155
Margaric acid (C17:0)	2.314 ± 0.018	2.273 ± 0.011	2.085 ± 0.005
Stearic acid (C18:0)	8.934 ± 0.004	10.236 ± 0.021	8.907 ± 0.019
Arachidic acid (C20:0)	0.288 ± 0.008	0.311 ± 0.005	0.326 ± 0.001
Behenic acid (C22:0)	0.309 ± 0.001	0.324 ± 0.009	0.316 ± 0.006
Lignoceric acid (C24:0)	0.610 ± 0.013	0.954 ± 0.015	0.609 ± 0.016
∑SFA	47.546 ± 0.172 ^{d,1}	46.471 ± 0.101 ^{d,2}	44.419 ± 0.007 ^{d,3}
Myristoleic acid (C14:1)	0.154 ± 0.002	0.141 ± 0.001	0.049 ± 0.000
Palmitoleic acid (C16:1)	4.343 ± 0.009	3.192 ± 0.008	4.184 ± 0.021
Palmitolaidic acid (C16:1t)	n.d.	n.d.	n.d.
Oleic acid (C18:1)	7.250 ± 0.032	7.936 ± 0.006	7.920 ± 0.013
Vaccenic acid (C18:1n7c)	2.924 ± 0.006	2.559 ± 0.001	3.191 ± 0.016
Eicosenoic acid (C20:1)	0.369 ± 0.013	0.405 ± 0.012	0.498 ± 0.006
Erucic acid (C22:1)	0.095 ± 0.003	0.096 ± 0.006	0.090 ± 0.008
Nervonic acid (C24:1)	0.307 ± 0.008	0.437 ± 0.010	0.461 ± 0.009
∑MUFA	15.440 ± 0.062 ^{g,2}	14.765 ± 0.006 ^{g,3}	16.931 ± 0.008 ^{g,1}
Linoleic acid (n-6, C18:2)	5.796 ± 0.068	4.805 ± 0.002	2.846 ± 0.032
γ -Linolenic acid (n-6, C18:3n6)	1.059 ± 0.004	0.844 ± 0.003	0.581 ± 0.006
α-Linolenic acid (n-3, C18:3n3)	4.649 ± 0.012	3.286 ± 0.009	5.998 ±0.012
Eicosadienoic acid (n-6, C20:2)	0.606 ± 0.009	0.466 ± 0.004	0.189 ± 0.016
Eicosatrienoic acid (n-6, C20:3n6)	0.859 ± 0.013	1.023 ± 0.005	0.312 ± 0.018
Arachidonic acid (n-6, C20:4)	3.451 ± 0.002	4.515 ± 0.013	3.832 ± 0.016
Ecosatrienoic acid (n-3, C20:3n3)	0.562 ± 0.014	0.439 ± 0.011	0.282 ± 0.000
Eicosapentaenoic acid, EPA (n-3, C20:5)	4.125 ± 0.008	3.837 ± 0.026	7.469 ± 0.010
Docosadienoic acid (n-6, C22:2)	0.012 ± 0.003	0.019 ± 0.005	0.014 ± 0.010
Adrenic acid (n-6, C22:4)	0.222 ± 0.002	0.236 ± 0.002	0.165 ± 0.001
Docosapentaenoic acid (n-6, C22:5n6)	2.177 ± 0.010	2.508 ± 0.023	2.964 ± 0.001
Docosapentaenoic acid, DPA (n-3, C22:5n3)	1.096 ± 0.002	1.156 ± 0.009	1.570 ± 0.005
Docosahexanoic acid, DHA (n-3, C22:6n3)	12.404 ± 0.006	15.636 ± 0.021	12.973 ± 0.009
∑PUFA	37.015 ± 0.110 ^{a,3}	38.766 ± 0.093 ^{a,2}	39.191 ± 0.016 ^{a,1}
UFA	52.454 ± 0.171 ^{bc,3}	53.530 ± 0.099 ^{bc,2}	55.582 ± 0.008 ^{bc,1}
∑n-3	22.834 ± 0.023 ^{b,3}	24.352 ± 0.076 ^{b,2}	28.291 ± 0.016 ^{b,1}
∑n-6	10.730 ± 0.085 ^{b,1}	9.899 ± 0.030 ^{b,2}	7.069 ± 0.015 ^{b,3}
n-3/n-6	2.128 ± 0.015 ^{e,3}	2.460 ± 0.000 ^{e,2}	4.002 ± 0.006 ^{e,1}
PUFA/SFA	0.779 ± 0.005 ^{a,3}	0.834 ± 0.004 ^{a,2}	0.882 ± 0.000 ^{a,1}

Table 8. - *contd.*

Fatty acid	FM-S	FM-D	FM-M
Caprylic acid (C8:0)	n.d.	0.004 ± 0.001	0.006 ± 0.002
Capric acid (C10:0)	0.013 ± 0.001	0.010 ± 0.000	0.010 ± 0.001
Lauric acid (C12:0)	0.493 ± 0.010	0.456 ± 0.004	0.257 ± 0.000
Myristic acid (C14:0)	2.199 ± 0.002	1.843 ± 0.022	2.240 ± 0.018
Pentadecylic acid (C15:0)	1.062 ± 0.019	1.025 ± 0.030	1.303 ± 0.016
Palmitic acid (C16:0)	26.270 ± 0.093	26.002 ± 0.302	27.267 ± 0.195
Margaric acid (C17:0)	1.595 ± 0.021	1.595 ± 0.030	1.992 ± 0.000
Stearic acid (C18:0)	9.442 ± 0.019	10.123 ± 0.129	10.427 ± 0.011
Arachidic acid (C20:0)	0.363 ± 0.001	0.357 ± 0.006	0.414 ± 0.007
Behenic acid (C22:0)	0.187 ± 0.006	0.152 ± 0.007	0.184 ± 0.008
Lignoceric acid (C24:0)	0.118 ± 0.008	0.088 ± 0.002	0.104 ± 0.005
∑SFA	41.739 ± 0.158 ^{e,2}	44.202 ± 0.199 ^{e,1}	41.652 ± 0.532 ^{e,2}
Myristoleic acid (C14:1)	0.070 ± 0.001	0.034 ± 0.004	0.046 ± 0.001
Palmitoleic acid (C16:1)	5.189 ± 0.072	3.641 ± 0.011	4.592 ± 0.005
Palmitolaidic acid (C16:1t)	n.d.	n.d.	n.d.
Oleic acid (C18:1)	28.732 ± 0.025	34.063 ± 0.229	32.302 ± 0.095
Vaccenic acid (C18:1n7c)	2.298 ± 0.016	2.274 ± 0.035	2.699 ± 0.008
Eicosenoic acid (C20:1)	0.747 ± 0.005	0.872 ± 0.022	0.759 ± 0.016
Erucic acid (C22:1)	0.031 ± 0.002	0.023 ± 0.004	0.019 ± 0.001
Nervonic acid (C24:1)	0.084 ± 0.019	0.049 ± 0.013	0.081 ± 0.000
∑MUFA	37.150 ± 0.059 ^{a,3}	40.496 ± 0.093 ^{a,2}	40.995 ± 0.148 ^{a,1}
Linoleic acid (n-6, C18:2)	9.914 ± 0.052	7.728 ± 0.325	7.243 ± 0.062
γ -Linolenic acid (n-6, C18:3n6)	0.522 ± 0.027	0.785 ± 0.086	0.351 ± 0.013
α-Linolenic acid (n-3, C18:3n3)	3.532 ± 0.007	2.646 ± 0.010	2.658 ± 0.046
Eicosadienoic acid (n-6, C20:2)	0.428 ± 0.001	0.424 ± 0.004	0.353 ± 0.013
Eicosatrienoic acid (n-6, C20:3n6)	0.478 ± 0.004	0.420 ± 0.003	0.333 ± 0.011
Arachidonic acid (n-6, C20:4)	2.082 ± 0.016	1.904 ± 0.006	1.689 ± 0.042
Ecosatrienoic acid (n-3, C20:3n3)	0.203 ± 0.010	0.147 ± 0.001	0.129 ± 0.014
Eicosapentaenoic acid, EPA (n-3, C20:5)	0.766 ± 0.006	0.643 ± 0.001	0.546 ± 0.018
Docosadienoic acid (n-6, C22:2)	0.013 ± 0.001	0.009 ± 0.000	0.008 ± 0.001
Adrenic acid (n-6, C22:4)	0.327 ± 0.006	0.340 ± 0.001	0.265 ± 0.006
Docosapentaenoic acid (n-6, C22:5n6)	0.551 ± 0.012	0.519 ± 0.005	0.371 ± 0.001
Docosapentaenoic acid, DPA (n-3, C22:5n3)	0.548 ± 0.002	0.455 ± 0.016	0.369 ± 0.011
Docosahexanoic acid, DHA (n-3, C22:6n3)	1.751 ± 0.001	1.377 ± 0.003	0.990 ± 0.040
∑PUFA	21.112 ± 0.102 ^{e,1}	15.302 ± 0.110 ^{e,3}	17.394 ± 0.389 ^{e,2}
UFA	58.262 ± 0.161 ^{a,1}	55.797 ± 0.202 ^{a,2}	58.349 ± 0.537 ^{a,1}
∑n-3	6.799 ± 0.013 ^{f,1}	4.691 ± 0.049 ^{f,3}	5.267 ± 0.023 ^{f,2}
∑n-6	12.231 ± 0.099 ^{a,1}	8.922 ± 0.103 ^{a,3}	10.224 ± 0.419 ^{a,2}
n-3/n-6	0.556 ± 0.006 ^{g,1}	0.526 ± 0.001 ^{g,1}	0.516 ± 0.023 ^{g,1}
PUFA/SFA	0.506 ± 0.004 ^{c,1}	0.346 ± 0.004 ^{c,3}	0.418 ± 0.015 ^{c,2}

Table 8. - *contd.*

Fatty acid	FA-S	FA-D	FA-M
Caprylic acid (C8:0)	n.d.	n.d.	n.d.
Capric acid (C10:0)	0.007 ± 0.001	0.011 ± 0.001	0.008 ± 0.001
Lauric acid (C12:0)	0.261 ± 0.006	0.336 ± 0.001	0.367 ± 0.002
Myristic acid (C14:0)	4.681 ± 0.006	5.524 ± 0.059	4.440 ± 0.019
Pentadecylic acid (C15:0)	0.781 ± 0.001	0.968 ± 0.001	0.681 ± 0.000
Palmitic acid (C16:0)	30.963 ± 0.119	32.517 ± 0.028	31.897 ± 0.060
Margaric acid (C17:0)	1.118 ± 0.001	1.274 ± 0.003	0.968 ± 0.003
Stearic acid (C18:0)	11.372 ± 0.010	11.261 ± 0.008	10.190 ± 0.005
Arachidic acid (C20:0)	0.772 ± 0.001	0.701 ± 0.005	0.636 ± 0.013
Behenic acid (C22:0)	0.754 ± 0.006	0.731 ± 0.002	0.641 ± 0.013
Lignoceric acid (C24:0)	0.703 ± 0.076	0.684 ± 0.033	0.401 ± 0.004
∑SFA	51.409 ± 0.037 ^{b,2}	50.226 ± 0.035 ^{b,3}	54.005 ± 0.002 ^{b,1}
Myristoleic acid (C14:1)	0.017 ± 0.001	0.025 ± 0.002	0.016 ± 0.001
Palmitoleic acid (C16:1)	5.497 ± 0.010	7.440 ± 0.080	7.111 ± 0.008
Palmitolaidic acid (C16:1t)	0.220 ± 0.013	0.282 ± 0.034	0.187 ± 0.003
Oleic acid (C18:1)	13.768 ± 0.106	14.819 ± 0.006	16.817 ± 0.060
Vaccenic acid (C18:1n7c)	3.341 ± 0.012	3.518 ± 0.042	3.399 ± 0.010
Eicosenoic acid (C20:1)	0.666 ± 0.010	0.477 ± 0.002	0.461 ± 0.002
Erucic acid (C22:1)	0.168 ± 0.015	0.070 ± 0.002	0.058 ± 0.007
Nervonic acid (C24:1)	0.705 ± 0.033	0.658 ± 0.016	0.414 ± 0.017
∑MUFA	24.381 ± 0.080 ^{d,3}	28.461 ± 0.062 ^{d,1}	27.287 ± 0.101 ^{d,2}
Linoleic acid (n-6, C18:2)	1.858 ± 0.011	2.584 ± 0.021	1.013 ± 0.008
γ -Linolenic acid (n-6, C18:3n6)	0.318 ± 0.006	0.395 ± 0.001	0.492 ± 0.013
α-Linolenic acid (n-3, C18:3n3)	0.625 ± 0.017	1.189 ± 0.011	0.489 ± 0.002
Eicosadienoic acid (n-6, C20:2)	0.232 ± 0.001	0.217 ± 0.000	0.187 ± 0.001
Eicosatrienoic acid (n-6, C20:3n6)	0.256 ± 0.003	0.214 ± 0.004	0.174 ± 0.003
Arachidonic acid (n-6, C20:4)	2.618 ± 0.005	1.684 ± 0.011	1.942 ± 0.016
Ecosatrienoic acid (n-3, C20:3n3)	0.120 ± 0.001	0.142 ± 0.004	0.111 ± 0.005
Eicosapentaenoic acid, EPA (n-3, C20:5)	4.410 ± 0.054	2.998 ± 0.013	4.948 ± 0.034
Docosadienoic acid (n-6, C22:2)	0.013 ± 0.004	0.013 ± 0.001	0.018 ± 0.004
Adrenic acid (n-6, C22:4)	0.527 ± 0.000	0.237 ± 0.004	0.343 ± 0.006
Docosapentaenoic acid (n-6, C22:5n6)	1.036 ± 0.008	0.585 ± 0.007	0.742 ± 0.004
Docosapentaenoic acid, DPA (n-3, C22:5n3)	1.736 ± 0.005	1.122 ± 0.008	2.108 ± 0.016
Docosahexanoic acid, DHA (n-3, C22:6n3)	10.462 ± 0.032	7.331 ± 0.135	8.749 ± 0.045
∑PUFA	24.209 ± 0.044 ^{d,1}	21.312 ± 0.099 ^{d,2}	18.709 ± 0.097 ^{d,3}
UFA	48.590 ± 0.036 ^{de,2}	49.773 ± 0.037 ^{de,1}	45.995 ± 0.004 ^{de,3}
∑n-3	17.352 ± 0.043 ^{d,1}	16.404 ± 0.087 ^{d,2}	12.781 ± 0.100 ^{d,3}
∑n-6	4.240 ± 0.006 ^{c,1}	2.967 ± 0.004 ^{c,2}	4.245 ± 0.013 ^{c,1}
n-3/n-6	4.092 ± 0.005 ^{d,2}	5.529 ± 0.037 ^{d,1}	3.011 ± 0.033 ^{d,3}
PUFA/SFA	0.471 ± 0.001 ^{c,1}	0.424 ± 0.002 ^{c,2}	0.346 ± 0.002 ^{c,3}

BD: Bombay duck (*Harpadon nehereus*); RF: ribbon fish (*Trichiurus iepturus*); WS: white sardine (*Escualosa thoracata*); FB: freshwater barb (*Puntius spp.*); GR: Ganges River sprat (*Corica soborna*); FM: fermented barb (*Puntius spp.*); FA: fermented anchovies (*Setipnna spp.*); C: Cox's Bazar; D: Dhaka; M: Mymensingh; S: Sylhet. SFA: saturated fatty acid; PUFA: polyunsaturated fatty acid; MUFA: monounsaturated fatty acid; n.d.: not detected. Different letters (a, b, and c) represent significant differences between fish types via two-way ANOVA (p<0.05). Different numbers (1, 2, and 3) represent significant differences within the same fish type and different locations via one-way ANOVA (p<0.05).

3.3.9 Cholesterol content

Animal-based foods are the only sources of dietary cholesterol, so it is naturally present in our diet and tissues. It 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, depending on type and sampling location (Table 6). GR and WS had the highest cholesterol levels (7.47 ± 0.01 - 13.55 ± 0.23 mg/g) while FM (4.20 ± 0.06 - 5.43 ± 0.07 mg/g) contained the lowest amounts. 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 contents obtained in the current study are due to the drying process, which concentrates cholesterol in the DFs. In addition to the fishing season, the eating habits and maturity of the fish also significantly affect the cholesterol content (Garcia-Vaquero et al., 2021). In the current study, cholesterol content of fermented DFs (FM) were found to be significantly lower than those of the unfermented FB (5.63 ± 0.10 - 5.94 ± 0.61 mg/g), which are consistent with the drop in cholesterol content of salted-fermented hoki roe (Bekhit et al., 2018).

The previous dietary guidance of >1 but <300 mg/day cholesterol for lowering cardiovascular disease risk have 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 in DFs, especially sun-dried fishes (Qiu et al., 2019), an additional fermentation process may contribute to the cholesterol content reduction and thereby reduce the COP content.

3.3.10 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 (0.171 ± 0.006 - 0.245 ± 0.00 µg/g) had the highest Vitamin B_{12} content, followed by RF with a range of 0.051 ± 0.000 - 0.149 ± 0.008 µg/g (Table 6). The findings in the current study are higher than the results reported in previous studies of Bombay duck with 0.015 µg/g of fresh fish (Nordhagen et al., 2020) and 0.036 µg/g for fresh Ganges River sprat (Bogard et al., 2015b).

The higher contents in DFs 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, at least 50% of which can be met by the consumption of 100 g of DFs used in this study. A higher adequate intake of vitamin B_{12} of 4.5 and 5.0 μg/day for pregnant women and lactating women was suggested (Obeid et al., 2019), and this can be met in full by consuming 100 g of RF, WS, FB, and GR. Overall, DFs can be strongly recommended as an excellent dietary source of vitamin B_{12} to prevent deficiency.

3.4 Conclusions

This chapter covers the macro- and micro-nutrient composition of seven types of Bangladesh DFs collected from four cities. Among these samples, two are medium/large fishes, three are indigenous small fishes and two are fermented small fish. In this study, the two indigenous small fishes, WS and GR, can contribute significantly more than other DFs in meeting the daily dietary requirements of calcium, potassium, manganese, iron, copper, zinc, EAA, EPA, DHA and vitamin B_{12} in vulnerable populations with negligible concerns about overdose. FB (another indigenous small fish) can also contribute to daily nutritional requirements in terms of multiple nutrients, however, its excessive sodium content, low protein content and quality, low vitamin B_{12} content and less preferable fatty acids profile when compared with WS and GR may make it less suitable for consumers' health. Medium/large DFs performed moderately in terms of daily nutritional contribution, that is, they contain moderate levels of various nutrients, but high cholesterol and high SFA content partially obscure the benefits of eating them. Fermented small fishes contained high level of ash,

especially sodium, calcium, manganese, and chromium, which are the elements that require caution as excessive intake is potentially harmful. In addition, the lipid degradation caused by fermentation could lead to an undesirable fatty acids profile but better protein digestibility. The significantly lower cholesterol and higher FAA contents in fermented DFs may be favorable to consumer acceptance. Considering the contents of heavy metals and various nutrients in current study, small fishes, especially WS, could be an important tool to alleviate malnutrition among vulnerable groups and enhance food security. In addition, considering the high protein content in DFs, potential use for protein extract production to develop functional food additives such as emulsifiers, foaming agents, gelling agents, or using proteolysis technology to develop health beneficial short peptides may lead to enhanced value addition. It should be noted that all the DFs used in this study, except WS and FA, were found to have excessive levels of heavy metals to varying degrees, which may not only be due to the concentration effect of drying, but may come from water pollution and irregular transportation and storage. However, to ensure food safety, the contaminants that need to be monitored should not be limited to heavy metals, but also include chemical residues (pesticides), foreign matter (sand), microorganisms (pathogenic bacteria and fungi), and microplastics, which are commonly found in DFs.

REFERENCES

Agnew, U. M., & Slesinger, T. L. (2020). Zinc toxicity. *Europepmc*.

Ahmed, I., Jan, K., Fatma, S., & Dawood, M. A. O. (2022). Muscle proximate composition of various food fish species and their nutritional significance: A review. *Journal of Animal Physiology and Animal Nutrition*, *106*(3), 690–719. https://doi.org/10.1111/jpn.13711

Al Alawi, A. M., Al Badi, A., Al Huraizi, A., & Falhammar, H. (2021). *Magnesium: The recent research and developments* (pp. 193–218). https://doi.org/10.1016/bs.afnr.2021.01.001

Bassit, R. A., Sawada, L. A., Bacurau, R. F. P., Navarro, F., Martins, E., Santos, R. V. T., Caperuto, E. C., Rogeri, P., & Costa Rosa, L. F. B. P. (2002). Branched-chain amino acid supplementation and the immune response of long-distance athletes. *Nutrition*, *18*(5), 376–379. https://doi.org/10.1016/S0899-9007(02)00753-0

Beaudry, A. G., & Law, M. L. (2022). Leucine Supplementation in Cancer Cachexia: Mechanisms and a Review of the Pre-Clinical Literature. *Nutrients*, *14*(14), 2824. https://doi.org/10.3390/nu14142824

Bekhit, A. E.-D. A., Duncan, A., Bah, C. S. F., Ahmed, I. A. M., Al-Juhaimi, F. Y., & Amin, H. F. (2018). Impact of fermentation conditions on the physicochemical properties, fatty acid and cholesterol contents in salted-fermented hoki roe. *Food Chemistry*, *264*, 73–80. https://doi.org/10.1016/j.foodchem.2018.05.008

Bellisle, F. (1999). Glutamate and the UMAMI taste: sensory, metabolic, nutritional and behavioural considerations. A review of the literature published in the last 10 years. *Neuroscience & Biobehavioral Reviews*, *23*(3), 423–438. https://doi.org/10.1016/S0149-7634(98)00043-8

Bhat, Z. F., Morton, J. D., Bekhit, A. E.-D. A., Kumar, S., & Bhat, H. F. (2022). Non-thermal processing has an impact on the digestibility of the muscle proteins. *Critical Reviews in Food Science and Nutrition*, *62*(28), 7773–7800. https://doi.org/10.1080/10408398.2021.1918629

Bhowmik, S., Zakaria, M. A., Sarwar, Md. S., Shofi, S. B., Syduzzaman, Akter, F., Islam, Md. M., & Mamun, A.-A. (2022). Development and nutritional index of ready to use fish products (RUFPs) from small fish species: Future superfoods for consumers. *Applied Food Research*, *2*(1), 100111. https://doi.org/10.1016/j.afres.2022.100111

Bidlingmeyer, B. A., Cohen, S. A., & Tarvin, T. L. (1984). RAPID ANALYSIS OF AMINO ACIDS USING PRE-COLUMN DERIVATIZATION. *Journal of Chromatography*, *336*, 93–104.

Bird, R. P., & Eskin, N. A. M. (2021). *The emerging role of phosphorus in human health* (pp. 27–88). https://doi.org/10.1016/bs.afnr.2021.02.001

Black, R. E., Victora, C. G., Walker, S. P., Bhutta, Z. A., Christian, P., de Onis, M., Ezzati, M., Grantham-McGregor, S., Katz, J., Martorell, R., & Uauy, R. (2013). Maternal and child undernutrition and overweight in low-income and middle-income countries. *The Lancet*, *382*(9890), 427–451. https://doi.org/10.1016/S0140-6736(13)60937-X

Bogard, J. R., Thilsted, S. H., Marks, G. C., Wahab, Md. A., Hossain, M. A. R., Jakobsen, J., & Stangoulis, J. (2015a). Nutrient composition of important fish species in Bangladesh and potential contribution to recommended nutrient intakes. *Journal of Food Composition and Analysis*, *42*, 120–133. https://doi.org/10.1016/j.jfca.2015.03.002

Bogard, J. R., Thilsted, S. H., Marks, G. C., Wahab, Md. A., Hossain, M. A. R., Jakobsen, J., &

Stangoulis, J. (2015b). Nutrient composition of important fish species in Bangladesh and potential contribution to recommended nutrient intakes. *Journal of Food Composition and Analysis*, *42*, 120–133. https://doi.org/10.1016/j.jfca.2015.03.002

Briscoe, M. (2015). Determination of Heavy Metals in Food by Inductively Coupled Plasma–Mass Spectrometry: First Action 2015.01. *Journal of AOAC INTERNATIONAL*, *98*(4), 1113–1120. https://doi.org/10.5740/jaoac.int.2015.01

Campos, H., Baylin, A., & Willett, W. C. (2008). α-Linolenic Acid and Risk of Nonfatal Acute Myocardial Infarction. *Circulation*, *118*(4), 339–345. https://doi.org/10.1161/CIRCULATIONAHA.107.762419

Carson, J. A. S., Lichtenstein, A. H., Anderson, C. A. M., Appel, L. J., Kris-Etherton, P. M., Meyer, K. A., Petersen, K., Polonsky, T., & Van Horn, L. (2020). Dietary Cholesterol and Cardiovascular Risk: A Science Advisory From the American Heart Association. *Circulation*, *141*(3). https://doi.org/10.1161/CIR.0000000000000743

Codex Alimentarius Commission. (2011). *Joint FAO/WHO food standards programme codex committee on contaminants in foods*.

Coskuntuna, L., Gecgel, U., Yilmaz, I., Gecgel, U., & Dulger, G. C. (2015). Erratum to: Investigating Fatty Acid Composition of Various Meat and Offal Products from Turkey. *Journal of the American Oil Chemists' Society*, *92*(5), 667–667. https://doi.org/10.1007/s11746-015-2637-0

Dantas, N. M., de Oliveira, V. S., Sampaio, G. R., Chrysostomo, Y. S. K., Chávez, D. W. H., Gamallo, O. D., Sawaya, A. C. H. F., Torres, E. A. F. da S., & Saldanha, T. (2021). Lipid profile and high contents of cholesterol oxidation products (COPs) in different commercial

brands of canned tuna. *Food Chemistry*, *352*, 129334. https://doi.org/10.1016/j.foodchem.2021.129334

David, B. (2012a). Amino Acids Synthesized from Aspartate: Lysine, Methionine (and Cysteine), Threonine and Isoleucine. In *Amino Acid Metabolism* (pp. 225–277). Wiley. https://doi.org/10.1002/9781118357514.ch6

David, B. (2012b). Amino Acids Synthesized from Glutamate: Glutamine, Proline, Ornithine, Citrulline and Arginine. In *Amino Acid Metabolism* (pp. 157–223). Wiley. https://doi.org/10.1002/9781118357514.ch5

DesMarias, T. L., & Costa, M. (2019). Mechanisms of chromium-induced toxicity. *Current Opinion in Toxicology*, *14*, 1–7. https://doi.org/10.1016/j.cotox.2019.05.003

Deutsch, M. J. (1994). Vitamins and Other Nutrients. *Journal of AOAC INTERNATIONAL*, *77*(1), 156–157. https://doi.org/10.1093/jaoac/77.1.156

EFSA. (2010). Scientific opinion on dietary reference values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol. *EFSA Journal*, *8*(3). https://doi.org/10.2903/j.efsa.2010.1461

Fairweather-Tait, S., & Sharp, P. (2021). *Iron* (pp. 219–250). https://doi.org/10.1016/bs.afnr.2021.01.002

FAO. (2013). *The state of food and agriculture*. http://bit.ly/KAn84P

FAO Expert Consultation. (2011). *Dietary protein quality evaluation in human nutrition*. https://www.fao.org/ag/humannutrition/35978-02317b979a686a57aa4593304ffc17f06.pdf

Fiedler, J. L., Lividini, K., Drummond, E., & Thilsted, S. H. (2016). Strengthening the

contribution of aquaculture to food and nutrition security: The potential of a vitamin A-rich, small fish in Bangladesh. *Aquaculture*, *452*, 291–303. https://doi.org/10.1016/j.aquaculture.2015.11.004

Foloch, J., Lees, M., & Sloane Stanley, G. H. (1957). A simple method for the isolation and purification of total lipides from animal tissues. *The Journal of Biological Chemistry*, *226*(1), 497–509.

Food and Nutrition Board, & Institute of Medicine. (2001a). Copper. In *Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc*. National Academy of Medicine.

Food and Nutrition Board, & Institute of Medicine. (2001b). Iron. In *Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc* (pp. 290–393). National Academy of Medicine.

Food and Nutrition Board, & Institute of Medicine. (2001c). Manganese. In *Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc* (pp. 394–419). National Academy of Medicine.

Food and Nutrition Board, & Institute of Medicine. (2001d). Zinc. In *Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc* (pp. 442–501). National Academy of Medicine.

Fu, Z., & Xi, S. (2020). The effects of heavy metals on human metabolism. *Toxicology Mechanisms and Methods*, *30*(3), 167–176. https://doi.org/10.1080/15376516.2019.1701594

Garcia-Vaquero, M., Rajauria, G., Miranda, M., Sweeney, T., Lopez-Alonso, M., & O'Doherty, J. (2021). Seasonal variation of the proximate composition, mineral content, fatty acid profiles and other phytochemical constituents of selected brown macroalgae. *Marine Drugs*, *19*(4), 204. https://doi.org/10.3390/md19040204

Gehrke, C. W., Wall, L. L., Absheer, J. S., Kaiser, F. E., & Zumwalt, R. W. (1985). Sample Preparation for Chromatography of Amino Acids: Acid Hydrolysis of Proteins. *Journal of AOAC INTERNATIONAL*, *68*(5), 811–821. https://doi.org/10.1093/jaoac/68.5.811

Genchi, G., Sinicropi, M. S., Lauria, G., Carocci, A., & Catalano, A. (2020). The Effects of Cadmium Toxicity. *International Journal of Environmental Research and Public Health*, *17*(11), 3782. https://doi.org/10.3390/ijerph17113782

Goto, T., Shimamoto, S., Ohtsuka, A., & Ijiri, D. (2021). Analyses of free amino acid and taste sensor traits in egg albumen and yolk revealed potential of value-added eggs in chickens. *Animal Science Journal*, *92*(1). https://doi.org/10.1111/asj.13510

Haider, Md. N., Bhattacharjee, S., Shikha, F. H., & Hossain, I. (2021). Bacterial Count and Proximate Composition of an Indian sub-continental Freshwater Barb, Punti (*Puntius sophore*) and a Gangetic Catfish, Gulsha (*Mystus cavasius*) during Drying-up Process. *Journal of Aquatic Food Product Technology*, *30*(4), 474–483. https://doi.org/10.1080/10498850.2021.1896613

Hasan, J., Lima, R. A., & Shaha, D. C. (2021). Fisheries resources of Bangladesh: A review.

International Journal of Fisheries and Aquatic Studies, *9*(4), 131–138. https://doi.org/10.22271/fish.2021.v9.i4b.2532

Hashimoto, K., Watabe, S., KoNo, M., & Shiro, K. (1979). Muscle protein composition of sardine and mackerel. *Bulletin of the Japanese Society of Scientific Fisheries*, *45*(11), 1435–1441.

Hei, A. (2021). Mental health benefits of fish consumption. *Psychol Psychiatry*, *2*(2).

Hoque, Md. S., Tamanna, F., Hasan, Md. M., Al Banna, Md. H., Mondal, P., Prodhan, M. D. H., Rahman, Md. Z., & van Brakel, M. L. (2022). Probabilistic public health risks associated with pesticides and heavy metal exposure through consumption of common dried fish in coastal regions of Bangladesh. *Environmental Science and Pollution Research*, *29*(14), 20112–20127. https://doi.org/10.1007/s11356-021-17127-9

Horwitz, W. (1997). *Official methods of analysis of AOAC International. Volume I, agricultural chemicals, contaminants, drugs* (W. Horwitz, Ed.; Vol. 1). AOAC International. http://hdl.handle.net/10637/3158

Hossain, M. N., Jamil, M. G. M., Mia, M. M., Uddin, M. N., & Mansur, M. A. (2017). Studies on the proximate composition, quality and heavy metal concentration of two sun-dried marine fish (sun-dried Silver Pomfret and sun-dried Perch) of Cox's Bazar District of Bangladesh. *Journal of Environmental Science and Natural Resources*, *10*(1), 25–32.

Houston, M. C. (2011). Role of Mercury Toxicity in Hypertension, Cardiovascular Disease, and Stroke. *The Journal of Clinical Hypertension*, *13*(8), 621–627. https://doi.org/10.1111/j.1751-7176.2011.00489.x

Hsu, H. W., Vavak, D. L., Satterlee, L. D., & Miller, G. A. (1977). A MULTIENZYME

TECHNIQUE FOR ESTIMATING PROTEIN DIGESTIBILITY. *Journal of Food Science*, *42*(5), 1269–1273. https://doi.org/10.1111/j.1365-2621.1977.tb14476.x

ICDDRB, U. (2013). *National micronutrients status survey*.

Institute of Medicine (US) Standing Committee. (1997). *Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride*. National Academies Press. https://doi.org/10.17226/5776

Islam, S., Bhowmik, S., Majumdar, P. R., Srzednicki, G., Rahman, M., & Hossain, Md. A. (2021). Nutritional profile of wild, pond-, gher- and cage-cultured tilapia in Bangladesh. *Heliyon*, *7*(5), e06968. https://doi.org/10.1016/j.heliyon.2021.e06968

Kar, M., Hoq, Md. E., Islam, Md. S., Islam, Md. M., Meghla, N. T., Suravi, S., & Kabir, Md. H. (2020). Monitoring of Proximate Composition, Heavy Metal Concentrations and Pesticide Residues in Marine Dried Fish Available in the Coastal Region of Bangladesh. *Grassroots Journal of Natural Resources*, *3*(2), 30–41. https://doi.org/10.33002/nr2581.6853.03023

Khatun, N., Nayeem, J., deb, N., Hossain, S., & Kibria, Md. M. (2021). Heavy metals contamination: possible health risk assessment in highly consumed fish species and water of Karnafuli River Estuary, Bangladesh. *Toxicology and Environmental Health Sciences*, *13*(4), 375–388. https://doi.org/10.1007/s13530-021-00101-w

Kubra, K., Azam, M., & Hoque, Md. S. (2020). Fish drying and socio-economic condition of dried fish producers in the coastal region of Bangladesh. *Middle-East J. Sci. Res*, *28*(3), 182–192.

Landry, J., & Delhaye, S. (1992). Simplified procedure for the determination of tryptophan of foods and feedstuffs from barytic hydrolysis. *Journal of Agricultural and Food Chemistry*,

40(5), 776–779. https://doi.org/10.1021/jf00017a014

Lecerf, J.-M., & de Lorgeril, M. (2011). Dietary cholesterol: from physiology to cardiovascular risk. *British Journal of Nutrition*, *106*(1), 6–14. https://doi.org/10.1017/S0007114511000237

Li, P., Yin, Y.-L., Li, D., Woo Kim, S., & Wu, G. (2007). Amino acids and immune function. *British Journal of Nutrition*, *98*(2), 237–252. https://doi.org/10.1017/S000711450769936X

Liu, J., Mai, R., Liu, P., Guo, S., Yang, J., & Bai, W. (2023). Flavor Formation in Dry-Cured Fish: Regulation by Microbial Communities and Endogenous Enzymes. *Foods*, *12*(16), 3020. https://doi.org/10.3390/foods12163020

Lovern, J. A. (1950). Some causes of variation in the composition of fish oils. *J. Soc. Leather Trades Chemists*, *34*, 7–23.

Mandal, A. (2021). Value chain analysis of dry fish marketing in coastal belt of Bangladesh. *International Journal of Fisheries and Aquatic Studies*, *9*(4), 217–222. https://doi.org/10.22271/fish.2021.v9.i4c.2538

Markwell, M. A. K., Haas, S. M., Bieber, L. L., & Tolbert, N. E. (1978). A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. *Analytical Biochemistry*, *87*(1), 206–210. https://doi.org/10.1016/0003-2697(78)90586-9

Martins, A. C., Krum, B. N., Queirós, L., Tinkov, A. A., Skalny, A. V., Bowman, A. B., & Aschner, M. (2020). Manganese in the Diet: Bioaccessibility, Adequate Intake, and Neurotoxicological Effects. *Journal of Agricultural and Food Chemistry*, *68*(46), 12893–12903. https://doi.org/10.1021/acs.jafc.0c00641

Mehlenbacher, V. C., Sallee, E. M., Hopper, T. H., Link, W. E., Walker, R. O., & Firestone, D. (2009). *Official Methods and Recommended Practices of the AOCS* (3rd ed.). AOCS Press.

Mohd Khairi, I. N. B., Huda, N., Wan Abdullah, W. N., & Al-Kharki, A. F. M. (2014). Protein quality of fish fermented product: budu and rusip. *Asia Pacific Journal of Sustainable Agriculture, Food and Energy*, *2*(2), 17-22.

Mosallanezhad, Z., Jalali, M., Bahadoran, Z., Mirmiran, P., & Azizi, F. (2023). Dietary sodium to potassium ratio is an independent predictor of cardiovascular events: a longitudinal follow-up study. *BMC Public Health*, *23*(1), 705. https://doi.org/10.1186/s12889-023-15618-7

Nazir, D. J., & Magar, N. G. (1965). Chemical composition of Bombay ducks (Harpodon nehereus) and changes occurring in the nutritive value of dried Bombay ducks on storage. *Fishery Technology*, *2*(2), 170–179.

Nindrea, R. D., Aryandono, T., Lazuardi, L., & Dwiprahasto, I. (2019). Association of Dietary Intake Ratio of n-3/n-6 Polyunsaturated Fatty Acids with Breast Cancer Risk in Western and Asian Countries: A Meta-Analysis. *Asian Pacific Journal of Cancer Prevention*, *20*(5), 1321–1327. https://doi.org/10.31557/APJCP.2019.20.5.1321

NIPORT, M., & Macro, O. R. C. (2005). *Bangladesh demographic and health survey 2004. Dhaka: National Institute of Population Research and Training*.

Niu, J., Zhao, B., Guo, X., & Yin, T. (2019). Effects of Vacuum Freeze-Drying and Vacuum Spray-Drying on Biochemical Properties and Functionalities of Myofibrillar Proteins from Silver Carp. *Journal of Food Quality*, *2019*, 1–8. https://doi.org/10.1155/2019/9457835

Nordhagen, A., Rizwan, A. A. Md., Aakre, I., Moxness Reksten, A., Pincus, L. M., Bøkevoll,

A., Mamun, A., Haraksingh Thilsted, S., Htut, T., Somasundaram, T., & Kjellevold, M. (2020). Nutrient Composition of Demersal, Pelagic, and Mesopelagic Fish Species Sampled Off the Coast of Bangladesh and Their Potential Contribution to Food and Nutrition Security—The EAF-Nansen Programme. *Foods*, *9*(6), 730. https://doi.org/10.3390/foods9060730

Obeid, R., Heil, S. G., Verhoeven, M. M. A., van den Heuvel, E. G. H. M., de Groot, L. C. P. G. M., & Eussen, S. J. P. M. (2019). Vitamin B12 Intake From Animal Foods, Biomarkers, and Health Aspects. *Frontiers in Nutrition*, *6*. https://doi.org/10.3389/fnut.2019.00093

O'Donnell, M., Mente, A., Alderman, M. H., Brady, A. J. B., Diaz, R., Gupta, R., López-Jaramillo, P., Luft, F. C., Lüscher, T. F., Mancia, G., Mann, J. F. E., McCarron, D., McKee, M., Messerli, F. H., Moore, L. L., Narula, J., Oparil, S., Packer, M., Prabhakaran, D., … Yusuf, S. (2020). Salt and cardiovascular disease: insufficient evidence to recommend low sodium intake. *European Heart Journal*, *41*(35), 3363–3373. https://doi.org/10.1093/eurheartj/ehaa586

Paul, P. C., Reza, M. S., Islam, M. N., & Kamal, M. (2018). A review on dried fish processing and marketing in the coastal region of Bangladesh. *Research in Agriculture Livestock and Fisheries*, *5*(3), 381–390. https://doi.org/10.3329/RALF.V5I3.39587

Phillips, C. M., Kesse-Guyot, E., McManus, R., Hercberg, S., Lairon, D., Planells, R., & Roche, H. M. (2012). High Dietary Saturated Fat Intake Accentuates Obesity Risk Associated with the Fat Mass and Obesity-Associated Gene in Adults. *The Journal of Nutrition*, *142*(5), 824–831. https://doi.org/10.3945/jn.111.153460

Qiu, X., Chen, S., & Lin, H. (2019). Oxidative Stability of Dried Seafood Products during

Processing and Storage: A Review. *Journal of Aquatic Food Product Technology*, *28*(3), 329–340. https://doi.org/10.1080/10498850.2019.1581317

Quintaes, K. D., & Diez-Garcia, R. W. (2015). The importance of minerals in the human diet. In *Handbook of Mineral Elements in Food* (pp. 1–21). John Wiley & Sons, Ltd. https://doi.org/10.1002/9781118654316.ch1

Rabie, M., Simon-Sarkadi, L., Siliha, H., El-seedy, S., & El Badawy, A.-A. (2009). Changes in free amino acids and biogenic amines of Egyptian salted-fermented fish (Feseekh) during ripening and storage. *Food Chemistry*, *115*(2), 635–638. https://doi.org/10.1016/j.foodchem.2008.12.077

Rahman, M. A., Saifullah, M., & Islam, M. N. (2012). Fish powder in instant fish soup mix. *Journal of the Bangladesh Agricultural University*, *10*(452-2016–35550), 145–148.

Rakib, Md. R. J., Jolly, Y. N., Enyoh, C. E., Khandaker, M. U., Hossain, M. B., Akther, S., Alsubaie, A., Almalki, A. S. A., & Bradley, D. A. (2021). Levels and health risk assessment of heavy metals in dried fish consumed in Bangladesh. *Scientific Reports*, *11*(1), 14642. https://doi.org/10.1038/s41598-021-93989-w

Rana, M. M., Chakraborty, S. C., & Saeid, A. (2020). Comparative Studies of Nutritional, Microbial and Organoleptic Properties of Different Indigenous Dried Fish from Local Market in Bangladesh. *Advanced Journal of Chemistry-Section A*, *3*(3), 318–327. https://doi.org/10.33945/SAMI/AJCA.2020.3.9

Ratnaike, R. N. (2003). Acute and chronic arsenic toxicity. *Postgraduate Medical Journal*, *79*(933), 391–396. https://doi.org/10.1136/pmj.79.933.391

Rudel, L. L., & Morris, M. D. (1973). Determination of cholesterol using o-phthalaldehyde.

Journal of Lipid Research, *14*(3), 364–366. https://doi.org/10.1016/S0022-2275(20)36896-6

Sachse, B., Kolbaum, A. E., Ziegenhagen, R., Andres, S., Berg, K., Dusemund, B., Hirsch-Ernst, K. I., Kappenstein, O., Müller, F., Röhl, C., Lindtner, O., Lampen, A., & Schäfer, B. (2019). Dietary Manganese Exposure in the Adult Population in Germany—What Does it Mean in Relation to Health Risks? *Molecular Nutrition & Food Research*, *63*(16), 1900065. https://doi.org/10.1002/mnfr.201900065

Semedo Tavares, W. P., Dong, S., Yang, Y., Zeng, M., & Zhao, Y. (2018). Influence of cooking methods on protein modification and in vitro digestibility of hairtail (Thichiurus lepturus) fillets. *LWT*, *96*, 476–481. https://doi.org/10.1016/j.lwt.2018.06.006

Shah, A. K. M. A., Tokunaga, C., Ogasawara, M., Kurihara, H., & Takahashi, K. (2009). Changes in Chemical and Sensory Properties of *Migaki-Nishin* (Dried Herring Fillet) during Drying. *Journal of Food Science*, *74*(7). https://doi.org/10.1111/j.1750-3841.2009.01271.x

Shah, A. M., Wang, Z., & Ma, J. (2020). Glutamine Metabolism and Its Role in Immunity, a Comprehensive Review. *Animals*, *10*(2), 326. https://doi.org/10.3390/ani10020326

Skalny, A. V., Aschner, M., & Tinkov, A. A. (2021). *Zinc* (pp. 251–310). https://doi.org/10.1016/bs.afnr.2021.01.003

Sousa, L., Oliveira, M. M., Pessôa, M. T. C., & Barbosa, L. A. (2020). Iron overload: Effects on cellular biochemistry. *Clinica Chimica Acta*, *504*, 180–189. https://doi.org/10.1016/j.cca.2019.11.029

The State of World Fisheries and Aquaculture 2022. (2022). FAO.

https://doi.org/10.4060/cc0461en

Ullah, Md. R., Rahman, Md. A., Haque, Md. N., Sharker, Md. R., Islam, M. M., & Alam, Md. A. (2022). Nutritional profiling of some selected commercially important freshwater and marine water fishes of Bangladesh. *Heliyon*, *8*(10), e10825. https://doi.org/10.1016/j.heliyon.2022.e10825

Usydus, Z., Szlinder-Richert, J., & Adamczyk, M. (2009). Protein quality and amino acid profiles of fish products available in Poland. *Food Chemistry*, *112*(1), 139–145. https://doi.org/10.1016/j.foodchem.2008.05.050

Van Elswyk, M. E., Schake, L. S., & Hargis, P. S. (1991). Research note: evaluation of two extraction methods for the determination of egg yolk cholesterol. *Poultry Science*, *70*(5), 1258–1260. https://doi.org/10.3382/ps.0701258

van Ruth, S., Brouwer, E., Koot, A., & Wijtten, M. (2014). Seafood and Water Management. *Foods*, *3*(4), 622–631. https://doi.org/10.3390/foods3040622

Vikøren, L. A., Nygård, O. K., Lied, E., Rostrup, E., & Gudbrandsen, O. A. (2013). A randomised study on the effects of fish protein supplement on glucose tolerance, lipids and body composition in overweight adults. *British Journal of Nutrition*, *109*(4), 648–657. https://doi.org/10.1017/S0007114512001717

Visessanguan, W., Benjakul, S., Riebroy, S., & Thepkasikul, P. (2004). Changes in composition and functional properties of proteins and their contributions to Nham characteristics. *Meat Science*, *66*(3), 579–588. https://doi.org/10.1016/S0309-1740(03)00172-4

Wani, A. L., Ara, A., & Usmani, J. A. (2015). Lead toxicity: a review. *Interdisciplinary Toxicology*, *8*(2), 55–64. https://doi.org/10.1515/intox-2015-0009

Weaver, C. M. (2013). Potassium and Health. *Advances in Nutrition*, *4*(3), 368S-377S. https://doi.org/10.3945/an.112.003533

Wendelaar Bonga, S. E., & Flik, G. (1993). *Calcium regulation in fish* (pp. 47–59). https://doi.org/10.1029/CE043p0047

Werner, T., Kumar, R., Horvath, I., Scheers, N., & Wittung-Stafshede, P. (2018). Abundant fish protein inhibits α-synuclein amyloid formation. *Scientific Reports*, *8*(1), 5465. https://doi.org/10.1038/s41598-018-23850-0

WHO, & UNICEF. (2017). *Report of the fourth meeting of the WHO-UNICEF technical expert advisory group on nutrition monitoring (TEAM)*.

Wood, J. D., Enser, M., Fisher, A. V., Nute, G. R., Sheard, P. R., Richardson, R. I., Hughes, S. I., & Whittington, F. M. (2008). Fat deposition, fatty acid composition and meat quality: A review. *Meat Science*, *78*(4), 343–358. https://doi.org/10.1016/j.meatsci.2007.07.019

World Health Organization. (2012). *Guideline: Sodium Intake for Adults and Children.*

Wu, G. (2009). Amino acids: metabolism, functions, and nutrition. *Amino Acids*, *37*(1), 1–17. https://doi.org/10.1007/s00726-009-0269-0

Wu, G., Meininger, C. J., McNeal, C. J., Bazer, F. W., & Rhoads, J. M. (2021). *Role of L-Arginine in Nitric Oxide Synthesis and Health in Humans* (W. Guoyao, Ed.; pp. 167–187). Springer Chem. https://doi.org/10.1007/978-3-030-74180-8_10

Yang, F., Rustad, T., Xu, Y., Jiang, Q., & Xia, W. (2015). Endogenous proteolytic enzymes – A study of their impact on cod (Gadus morhua) muscle proteins and textural properties in a fermented product. *Food Chemistry*, *172*, 551–558. https://doi.org/10.1016/j.foodchem.2014.09.086

Yang, Q., Zhao, D., Zhang, C., Sreenivasulu, N., Sun, S. S.-M., & Liu, Q. (2022). Lysine biofortification of crops to promote sustained human health in the 21st century. *Journal of Experimental Botany*, *73*(5), 1258–1267. https://doi.org/10.1093/jxb/erab482

Yin, M., Matsuoka, R., Yanagisawa, T., Xi, Y., Zhang, L., & Wang, X. (2022). Effect of different drying methods on free amino acid and flavor nucleotides of scallop (patinopecten yessoensis) adductor muscle. *Food Chemistry*, *396*, 133620. https://doi.org/10.1016/j.foodchem.2022.133620

Zaman, M., Naser, M. N., Abdullah, A. T. M., & Khan, N. (2014). Nutrient contents of some popular freshwater and marine fish species of Bangladesh. *Bangladesh Journal of Zoology*, *42*(2), 251–259.

Zhao, C. J., Schieber, A., & Gänzle, M. G. (2016). Formation of taste-active amino acids, amino acid derivatives and peptides in food fermentations – A review. *Food Research International*, *89*, 39–47. https://doi.org/10.1016/j.foodres.2016.08.042

Zhen, Y., Ge, L., Chen, Q., Xu, J., Duan, Z., Loor, J. J., & Wang, M. (2022). Latent Benefits and Toxicity Risks Transmission Chain of High Dietary Copper along the Livestock–Environment–Plant–Human Health Axis and Microbial Homeostasis: A Review. *Journal of Agricultural and Food Chemistry*, *70*(23), 6943–6962. https://doi.org/10.1021/acs.jafc.2c01367

TRANSITION STATEMENT

Dried fishes (DFs) are rich in protein and widely available world-wide, however it has long been limited to being used as a traditional food rather than an innovative food ingredient. The next chapter focuses on the functional properties of dried fish protein isolates (DFPIs) prepared using isoelectric precipitation method to broaden the application scope of DF in the food industry. This study was conducted under the assumption that DFPIs will have altered protein structural properties and functionalities including solubility, emulsification, foaming, gelation, water and oil holding capacity, and heat coagulability.

CHAPTER FOUR

4 STRUCTURAL AND FUNCTIONAL PROPERTIES OF DRIED FISH PROTEIN ISOLATES

Huan Sun [1], Derek S. Johnson [2], Rotimi E. Aluko [1, 3, *]

*Corresponding author. E-mail address: rotimi.aluko@umanitoba.ca (Rotimi E. Aluko)

1 Department of Food and Human Nutritional Sciences, University of Manitoba, Room 209 Human Ecology Building, 35 Chancellor's Circle, Winnipeg, MB R3T 2N2, Canada

2 Department of Anthropology, University of Manitoba, 432 Fletcher Argue Building, 15 Chancellor Circle, Winnipeg, MB R3T 2N2, Canada

3 Richardson Centre for Food Technology and Research, University of Manitoba, 196 Innovation Dr, Winnipeg, MB R3T 2N2, Canada

Contribution of Authors statements

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

4.1 Introduction

Fish is rich in protein, and therefore, it is easy to produce by-products that are not fully utilized under modern industrial production, which consequently causes accumulation of protein-rich waste (Gehring et al., 2011). Extracting proteins from fishery products and by-products and exploring their potential use in the food industry could improve economic efficiency and alleviate the dilemma of unsustainable animal-based foods due to overfishing (Gehring et al., 2011). Since fish protein isolate (FPI) has proven to be excellent in more than one aspect, for example, water retention capacity, gelling, foam stability and emulsion capacity, it is widely used in various food systems (Shaviklo, 2015). Multiple studies reported on the applications of FPI as a binder (related to the gelling ability) in reconstituted meat (Chung et al., 2000), and as an emulsifier (so that the water and oil do not stratify) in muscle foods (Ramírez et al., 1999). FPI has been used to formulate food products such as puffed corn snacks (Shaviklo, Olafsdottir, et al., 2011), ice cream (Shaviklo, Thorkelsson, et al., 2011), bread (Adeleke & Odedeji, 2010), biscuits (Ibrahim, 2009), mayonnaise (Sathivel et al., 2005), soup powder (Rahman et al., 2012; Reza Shaviklo, 2012), ready-to-use fish cluster mix (Shaviklo et al., 2013), sausages (Surasani et al., 2022), and weaning foods (Hussain et al., 2007). In addition, FPI can serve as a binder for animal feed (Shaviklo & Etemadian, 2019), as well as formation of edible films or coatings for fried foods (Pires et al., 2012).

Dried fishes (DFs) are considered as a product of the fishery industry, having a long history for thousands of years (Belton et al., 2022). DFs are widely consumed around the world, especially in East Asia, South Asia, India, and Africa, where they are deeply loved by local people as an important part of traditional diets (Belton et al., 2022). However, like other by-

products from fisheries, most uses for DFs are currently limited to traditional cooking methods (Banna et al., 2022). Moreover, its potential to produce FPI has long been overlooked. Although some studies have explored DFs as a super supplement to alleviate micronutrient malnutrition (Bhowmik et al., 2022), and others have explored optimizing the production process to improve quality (Hamdani et al., 2018; Nagwekar et al., 2017), these are still not enough to bridge the gap with respect to value added utilization of fish proteins. One of the reasons is that some DFs have strong local characteristics, they are not well known globally, and have not been valued for a long time; therefore, many of them remain unstudied.

The structure of each the fish protein fractions have been well explained (Ochiai & Ozawa, 2020). As described by Hashimoto et al. (1979), fish muscle proteins include water-soluble sarcoplasmic proteins (20–50%), salt-soluble myofibrillar proteins (50–70%), and the insoluble matrix proteins (~3%). Components of these proteins also contain different subspecies of proteins. For example, sarcoplasmic proteins contain glycolytic enzymes, creatine kinase, myoglobin, and parvalbumin; myofibrillar proteins contain myosin, actin, tropomyosin, troponin, and paramyosin (in many invertebrate species), whereas matrix proteins are primarily composed of extracellular matrix proteins such as collagen. Studies have been published on exploiting the functionality of fish proteins. Feng et al. (2023) reported that freeze-thaw stable gel made from fish myofibrils and myofibrillar protein can improve the freeze-thaw stability of food products and ensure the quality of frozen food. Xiong et al. (2019) reported the formation of a stable emulsion with myofibrillar protein (and xanthan gum) after sonication, which can be used as a new delivery system for functional materials. Ding et al. (2022) described adding sarcoplasmic proteins to surimi to improve the hardness and elasticity

of surimi colloid as well as increasing water holding capacity.

Fishery is a pillar industry of Bangladesh's economy, which has a deep and extensive background in fish processing, resulting in abundant DFs that are unstudied (Shamsuzzaman et al., 2020). Previous researchers have confirmed that Bangladeshi DFs contain more than 50% protein content (Banna et al., 2022), which lays a realistic foundation for the production of dried fish protein isolates (DFPIs). However, there is scant information on the physicochemical and functional characteristics of DFPIs, especially their potential to serve as food ingredients. Therefore, the purpose of current investigation was to determine the structure (polypeptide composition, surface hydrophocity and conformation) and function (solubility, heat coagulation, water/oil holding capacity, gelling properties, emulsifying properties) of DFPIs prepared from DFs that were obtained from local markets in Dhaka, Bangladesh.

4.2 Materials and methods

4.2.1 Raw material preparation

Seven different types of dried fish, namely Bombay duck (BD, *Harpadon nehereus*), ribbon fish (RF, *Trichiurus lepturus*), white sardine (WS, *Escualosa thoracata*), freshwater barb (FB, *Puntius spp.*), Ganges River spar (gr, *Corica soborna*), fermented barb (FM, *Puntius spp.*), and fermented anchovies (FA, *Setipnna spp.*) were purchased from local markets in Dhaka, Bangladesh and then transported to the laboratory. Upon arrival, the samples were stored at -20°C until used for the experiments. All chemical reagents used in this study were of analytical grade and purchased from Sigma-Aldrich (Saint Louis, Missouri, USA) and Fisher Scientific Company (Oakville, ON, Canada). Double-distilled water (DDW) was used for reagent preparation to guarantee the accuracy and repeatability of the results.

4.2.2 Raw material preparation

The DFs were thawed at 4 ℃ overnight (12 h) and then dried in a 50℃ preheated oven for 24 h. The cooled-to-room-temperature DFs were immediately ground into dried fishmeal (DFM) and stored in airtight sample bottles to await defatting. DFMs were continuously defatted with acetone using a powder/acetone ratio of 1/10 (w/v) for 30 min. The mixture was then left to stand at room temperature until the supernatant was transparent and discarded. The defatting process was repeated three times, and DDFMs (defatted dried fishmeal) were spread evenly on a clean tray in a fume hood for 12 h to dry, followed by grinding and storage in tightly capped bottles at -20°C.

4.2.3 Dried fish protein isolates (DFPIs) extraction

The DDFM was mixed with DDW (5:100, w/v) and adjusted to pH 10 by 1 M NaOH addition, and then stirred continuously for 1 h. The mixture was centrifuged at 1600 g for 30 min, after which the supernatant was filtered with cheesecloth (grade 90, 40 x 36 thread count), adjusted to pH 4.5 using 1 M HCl, and stirred constantly for 30 min. The mixture was centrifuged, and precipitate collected, washed with DDW twice and then adjusted to pH 7.0 before freeze-drying. The final product dried fish protein isolates (DFPIs) were kept in airtight sample bottles at -20°C pending for further experiments.

4.2.4 Functional proprieties of DFPIs

4.2.4.1 Solubility

Solubility of DFPIs was determined by a previously published method with some modifications (Malomo et al., 2014). Ten mg protein of each sample was vortexed and hydrated thoroughly in 5 ml of 0.1 M phosphate buffer (pH 7.0) for 1 h. The resulting mixture was then

centrifuged at 1600 g for 30 min. The protein content of each supernatant was determined using the modified Lowry method (Markwell et al., 1978). The total protein content of each DFPI was determined by hydrating the sample with 0.1 M NaOH solution and following same steps as above mentioned. The solubility of each DFPI was calculated as follows:

DFPIs solubility (%) = (protein content of supernatant)/Total protein content of sample) ×100

4.2.4.2 Heat coagulability (HC)

Heat coagulability (HC) of the DFPIs was determined by a slightly modified method (Osemwota et al., 2021). Briefly, the DFPI protein solution (10 mg protein/ml phosphate buffer, pH 7.0) was heated at 100 ºC in a water bath for 15 min. Then the mixture was centrifuged (1600 g for 30 min) and the protein content (PC I) of the supernatant determined with the Lowry method (Markwell et al., 1978). Meanwhile, the total amount of protein (PC II) in the 10 mg protein/ml DFPI solutions (phosphate buffer, pH 7.0) was also determined by the same Lowry method. The HC of each DFPIs was calculated as follows:

DFPIs HC (%) = (PC II – PC I)/PC II ×100

4.2.4.3 Water and oil holding capacity

Water holding capacity (WHC) and oil holding capacity (OHC) of DFPIs were determined using a previously outlined method with some modifications (Malomo et al., 2014). One volume of 40 mg protein/ml liquid was prepared with phosphate buffer (pH 7.0) or pure canola

oil in pre-weighted 15 ml centrifuge tubes (empty tube + sample weight: WI, protein sample weight: WII). Samples were vortexed and then allowed to stand for 30 min at room temperature. The mixture was then centrifuged for 15 min at 1600 g, followed by draining of excess buffer or oil, after which the weight of the tube + residue was obtained as WIII. The WHC or OHC of each DFPIs was determined using the following equations.

$$\text{WHC (g of water/g of protein)} = (\text{WIII} - \text{WI})/\text{WII}$$

$$\text{OHC (ml of oil/g of protein)} = ((\text{WIII} - \text{WI})/0.92\ \text{g/ml})/\text{WII}$$

Where 0.92 g/ml is the density of pure canola oil

4.2.4.4 Least gelation concentration (LGC)

The LGC was determined by the method of Malomo et al. (2014). Different concentrations (2% to 20%, w/v, protein weight basis) of sample suspensions in DDW were thoroughly vortexed in 5 ml glass tubes and heated in a 95 °C water bath for 1 h. After rapidly cooling the tubes under tap water, the mixtures were then refrigerated (4 °C) for 14 h. The sample concentration at which the gel did not slip upon inverting the tube was taken as the LGC.

4.2.4.5 Emulsion formation and stability

Oil-in-water emulsions were prepared by homogenizing 5 ml of 10, 15, and 20 mg protein/ml (prepared in 0.1 M phosphate buffer, pH 7.0) with 1 ml of pure canola oil at 20,000 rpm for 2 min as described by Chao et al. (2018). The homogenizer (Polytron PT 10-35, Kinematica AG, Lucerne, Switzerland) was equipped with a 12-mm generator. The oil droplet size ($d_{3,2}$) of the emulsion was determined in a Mastersizer 3000 (Malvern Instruments Ltd.,

Malvern, U.K.) with distilled water as dispersant. The emulsified sample was added to the sample dispersion unit (Hydro 3000S, attached to the instrument) containing approximately 100 ml of water under constant shear until the desired level of obscuration is reached. The instrument was set to automatically measure the emulsion oil droplet size in five replicates, with each sample prepared in duplicate. The mean of the oil droplet size ($d_{3,2}$) of each sample was used as an indicator of emulsifying capacity (EC). The emulsified sample was then allowed to stand at room temperature for 30 min, and the oil droplet size distribution and average particle size ($d_{3,2}$) of each sample was measured again as an indicator to evaluate the emulsion stability (ES). ES was calculated as follows:

$$\text{ES} = \text{oil droplet size at 0 min } (d_{3,2}) / \text{ oil droplet size after 30 min } (d_{3,2})$$

4.2.5 Structural properties of DFPIs

4.2.5.1 Surface hydrophobicity (H_o)

H_o was determined using the method of Haskard and Li-Chan (1998), with 1-anilino-8-naphthalenesulfonate (ANS) as the probe. Solutions of each DFPI (10 mg protein/ml) were prepared by dissolving the sample in 10 mM phosphate buffer (pH 7.0). The solutions were then thoroughly vortexed and hydrated for 1 h at room temperature and then centrifuged at 11200 g for 10 min. The supernatants were diluted into a series of concentrations, ranging from 50 to 250 µg/ml, with 10 mM phosphate buffer (pH 7.0). A 5 µl aliquot of 8 mM ANS prepared in 10 mM phosphate buffer (pH 7.0) was added to every 200 µl of protein solution. A spectrofluorometer (JASCO, FP-6300) was set at an excitation wavelength of 390 nm and an

emission wavelength of 470 nm to measure the fluorescence intensity (FI) of each sample. H_o of each sample was calculated as the slope of the FI versus protein concentration plot.

4.2.5.2 Circular dichroism (CD)

The secondary and tertiary structures of the DFPIs were determined by obtaining the far- and near-UV spectra information on a spectropolarimeter (JASCO, J-810). Sample solutions (10 mg protein/ml) were prepared by thoroughly vortexing and hydrating DFPIs in 0.1 M phosphate buffer (pH 7.0), after which a 30 min, 11200 g centrifugation was applied to the suspensions to obtain a clear supernatant. The supernatants were then further diluted into 2 mg protein/ml and 6 mg protein/ml for far- and near-UV spectra measurements, respectively. The far-UV spectra were measured at 190-240 nm in a 0.05 cm path length cuvette, while the near-UV spectra were measured at 250–320 nm in a 0.1 cm path length cuvette. All CD spectra were obtained by calculating the average of three consecutive scans and subtracting the corresponding buffer spectra. The Far-UV data was analyzed using the SELCON3 algorithm (Whitmore & Wallace, 2004) located at DichroWeb (http://dichroweb.cryst.bbk.ac.uk/html/home.shtml, accessed 10 June 2023).

4.2.5.3 Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

The polypeptide composition of DFPIs was determined by conducting the SDS-PAGE on a Mini-Protein electrophoresis unit (Bio-Rad Laboratories, Inc., California, USA) according to the method described by Laemmli (1970) and Raikos et al. (2014) with slight modifications. The protein sample was dispersed in 5% sodium dodecyl sulfate (SDS) solution, heated in a water bath at 90 °C for 1 h, and then centrifuged (5000 g, 10 min) to obtain 6 mg protein/ml protein solution. An equal volume of Laemmli buffer was mixed with the protein solution to

obtain a protein concentration of 3 mg/ml (v/v) and was used as the non-reducing sample. Reducing samples were prepared with 2-mercaptoethanol (2-mercaptoethanol: Laemmli buffer: protein solution = 1:19:20, v/v/v) and incubated in a water bath at 90°C for 5 min. Five microliters of each prepared sample and 10 μl of protein standard were loaded onto a 4–15% Mini-Protean® TGX™ precast gel and run in a Mini-Protean II electrophoresis tank at 150 V for 1 hour. After staining (Coomassie Brilliant Blue R-250, 1 h) and destaining (50% methanol and 10% acetic acid aqueous solution, 2 h), the resulting gel was analyzed using a ImageQuant TL 1.0 imaging system (Cytiva, Montreal, CA) for band imaging.

4.2.6 Statistical analysis

Duplicate replications were used to obtain mean and standard deviations. Statical analysis was conducted on an IBM SPSS 28.0.0.0 (IBM, 2023) software with performing one-way ANOVA, taking Duncan's multiple range test ($p<0.05$).

4.3 Results and discussions

4.3.1 Protein content, yield and solubility of DFPIs

As shown in Figure 1, the isoelectric point (IP) of DFPIs is obviously inconsistent with the pH 5.5 reported in some previous articles (Álvarez et al., 2018; Chen & Jaczynski, 2007; Y. Tan & Chang, 2021). The main reason could be that a large amount of NaCl was added as a preservative when processing the DFs, consequently enhancing the overall ion strength of the extraction solution. The IP of pH 4.5 for DFPIs as reported in the current study is consistent with the pH 4.0-4.5 reported by Chen & Jaczynski (2007) when extracting rainbow trout (*Oncorhynchus mykiss*) by-products under the condition of ionic strength of 0.2 M NaCl. This can be further explained by the work of Aluko & Yada (1997), which reported that in an acidic

environment, proteins with protonated surface residues will be masked by Cl^-, resulting in a weakening of the protein-protein repulsion (protein aggregation). Thus, under high ionic strength (high concentration of Cl^-), more protonated protein surface residues are required to achieve protein aggregation, resulting in the IP moving towards a lower pH value.

In the current study, except from BD-D (44.70 ± 0.01%), all DFPIs were detected with a ≥70% protein content, which is in agreement with the description in Shaviklo (2015) of a general ≥65% of protein content, with the highest value (87.32 ± 0.41%) found in RF-D (Table 1). The protein content of BD-D obtained in present study is within the 36-53% range that was reported by Chen & Jaczynski (2007) for rainbow trout protein isolates. The yields of the DFPIs were varied among dried fish types, with the yield of BD-D (6.65 ± 1.33%) and FM-D (5.64 ± 0.00%) significantly lower than those of other DFs while GR-D (51.18 ± 0.29%) had significantly higher value than the other DFs. The protein yield of GR-D is consistent with the 46% protein recovery of silver carp (*Hypophthalmichthys molitrix*) when extracted at pH 10 (Zhong et al., 2016). Zhong et al. (2016), also stated that when extracted at an extreme pH value (pH 2 or 12) a higher yield was achievable. In this sense, when applying a higher extraction pH, a better protein yield could be possible. In addition to extraction conditions, factors such as processing treatments, storage conditions, oxidation level of the protein could also have affected yields of the DFPIs.

Solubility of DFPIs varied significantly according to DF types, with the highest value found in BD-D (85.03 ± 1.06%) and lowest in FA (9.35 ± 0.34%) as shown in Table 1. Previous reports have pointed out that in the range of pH 2-12, the plot of solubility against pH for most animal and plant proteins have shown a V-shaped distribution, with the lowest solubility being

the previously reported in the pH 5-6 range (Aluko & Yada, 1997; Álvarez et al., 2018; Chen & Jaczynski, 2007; Y. Tan & Chang, 2021; Zhong et al., 2016). In the range of pH 4-9, solubility of fish protein isolate (FPI) is relatively low and will significantly increase when pH value increases beyond that range (Rodrigues Freitas et al., 2016), which can well explain the lower solubility of DFPIs that we observed in WS-D, GR-D, and FA-D at pH 7.0. However, regarding the significantly higher solubility of BD-D and FM-D at pH 7.0, the possible explanation is that more water-soluble proteins were extracted during the extraction process due to a higher ionic strength (Chen & Jaczynski (2007) and the sample nature (containing more water-soluble proteins), leading to an increase in the solubility of the corresponding DFPIs at pH 7.0. It is also possible that the proteins in BD-D and FM-D did not experience severe adverse denaturing effects to their structures during processing.

Table 1. Protein contents, yields and solubility at pH 7.0 of dried fish protein isolates (DFPIs)

Sample ID	Protein content (%)	DFPIs yields	Solubility (%)
BD-D	44.70 ± 0.01 ^{g}	6.65 ± 1.33 ^{e}	85.03 ± 1.06 ^{a}
RF-D	87.32 ± 0.41 ^{a}	24.95 ± 1.47 ^{c}	58.90 ± 1.76 ^{d}
WS-D	81.64 ± 0.68 ^{b}	36.94 ± 1.34 ^{b}	28.13 ± 0.79 ^{e}
FB-D	69.57 ± 0.97 ^{f}	18.09 ± 1.42 ^{d}	68.53 ± 0.51 ^{c}
GR-D	77.39 ± 0.79 ^{d}	51.18 ± 0.29 ^{a}	28.36 ± 0.11 ^{e}
FM-D	78.60 ± 0.05 ^{c}	5.64 ± 0 ^{e}	72.71 ± 1.15 ^{b}
FA-D	73.65 ± 0.61 ^{e}	18.4 ± 1.27 ^{d}	19.35 ± 0.34 ^{f}

BD: Bombay duck (*Harpadon nehereus*); RB: ribbon fish (*Trichiurus iepturus*); WS: white sardine (*Escualosa thoracata*); FB: freshwater barb (*Puntius spp*); GR: Ganges River sprat (*Corica soborna*); FM: fermented barb (*Puntius spp*); FA: fermented anchovies (*Setipnna spp*); D: Dhaka; Different letters (a, b, and c) represent significant differences between fish types via one-way ANOVA.

4.3.2 Sodium dodecyl sulfhate-polyacrylamide gel electrophoresis (SDS-PAGE)

Figure 1 shows the distribution of polypeptide molecular weight (MW) of DFPIs at pH 7. It can be clearly found that no myosin heavy chain (MHC) was detected at 200 kDa in all DFPIs, and in addition, actin was also lacking at 48 kDa, which suggest that severe degradation occurred in myofibrillar proteins during dry fish processing (Wang et al., 2011). In a study of ham peptides MW distribution, which underwent similar treatments (salting and drying) as the dried fish, it was reported that heavy chains and actin were degraded into smaller peptide chains under the combined action of salt (Wang et al., 2017), endogenous muscle peptidases and microbial enzymes (Poljanec et al., 2021). In current study, the MW of DFPIs mainly showed broad bands at 25-37 kDa and 10-15 kDa, which are very similar to the broad bands at 14-20 kDa found in ripened ham sarcoplasmic proteins (Poljanec et al., 2021). The findings of 34 kDa and 25 kDa bands in the reducing gel are in agreement with the 34.9 and 25.6 kDa bands reported in ham's sarcoplasmic proteins (Poljanec et al., 2021). A similar finding was also

reported (Wang et al., 2017), indicating that with increasing fermentation duration, the intensity of the 35 kDa band increased in both sarcoplasmic and myofibrillar protein fractions in fermented fish products. This shows that DFPIs are complex protein mixtures, including not only hydrolyzed myofibrillar proteins but also parts of the sarcoplasmic proteins.

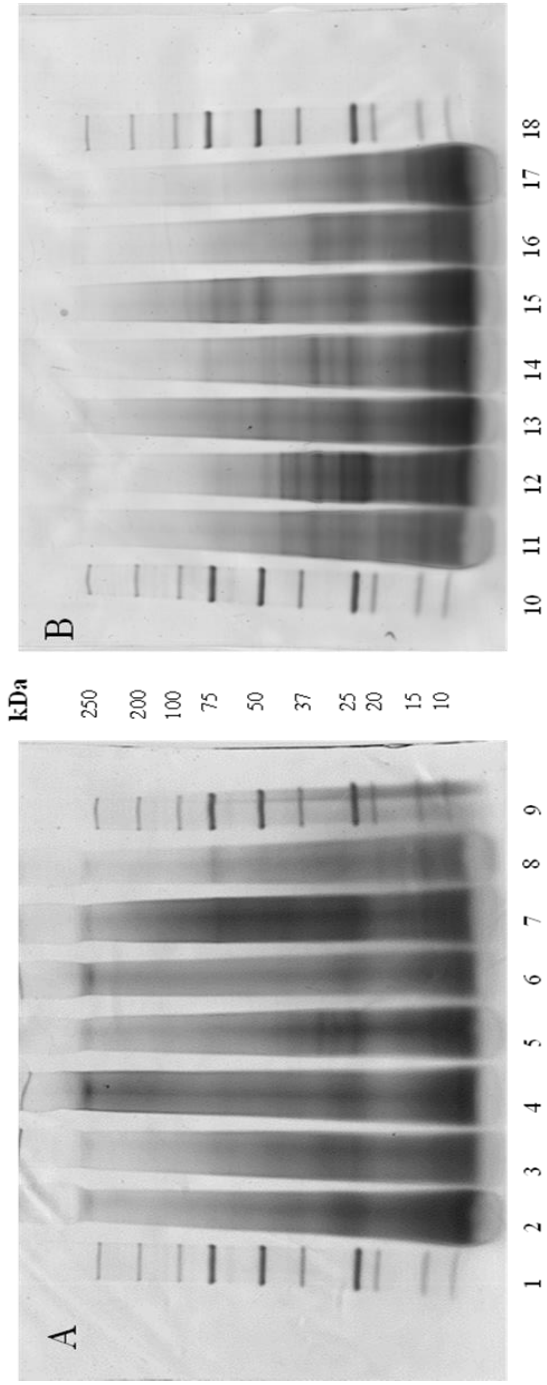

Figure 1. SDS-PAGE patterns of dried fish protein isolates (DFPIs). A: non-reducing gel; B: reducing gel. Lane 1, 9, 10, 18: molecular weight standards; lane: 8, 11: BD-D; lean 7, 12: RF-D; lane 6, 13: WS-D; lane 5, 14: FB-D; lane 4, 15: GR-D; lane 3, 16: FM-D; lane 2, 17: FA-D. BD: Bombay duck (*Harpadon nehereus*); RF: ribbon fish (*Trichiurus lepturus*); WS: white sardine (*Escualosa thoracata*); FB: freshwater barb (*Puntius spp)*; GR: Ganges River sprat (*Corica soborna*); FM: fermented barb (*Puntius spp*); FA: fermented anchovies (*Setipnna spp*); D: Dhaka.

4.3.3 Surface hydrophobicity (H_o)

BD-D had the highest H_o of about 13064.00 ± 185.26, which is significantly higher than those of other DFPIs (Table 2). A higher H_o indicates that BD-D has more exposed hydrophobic groups compared with other DFPIs, which suggests that BD-D proteins may have been more unfolded as a consequence to the manufactural processing. On the other hand, WS-D and GR-D had a significantly lower value, indicating that these DFPIs had a more folded conformation. Tadpitchayangkoon et al. (2010) reported a H_o of about 7000 at pH 7.0, for striped catfish (*Pangasius hypophthalmus*) sarcoplasmic proteins, which is slightly higher than the findings reported in our study, except for BD-D. Kobayashi & Park (2018) suggested that in fish protein isolate extracted with alkaline pH, there is a resultant lack of restoration of the myosin head structure, which leads to increased exposure of hydrophobic clusters. More importantly, effects from factors such as salting, drying, and microbial digestion will also accelerate protein degradation and lead to the exposure of more hydrophobic clusters, thereby affecting the H_o of DFPIs (Mohd Khairi et al., 2014).

Table 2. Heat coagulability, water holding capacity, oil holding capacity, least gelation concentration and surface hydrophobicity of dried fish protein isolates

Sample ID	Heat coagulability (%)	Water holding capacity (g/g)	Oil holding capacity (ml/g)	Least gelation concentration (%)	Surface Hydrophobicity
BD-D	6.23 ± 0.41 ^{bc}	7.00 ± 0.47 ^{a}	20.13 ± 0.14 ^{a}	3	13064.00 ± 185.26 ^{a}
RF-D	7.36 ± 1.26 ^{bc}	2.79 ± 0.04 ^{d}	15.38 ± 0.72 ^{c}	3	3900.85 ± 123.67 ^{d}
WS-D	6.60 ± 2.94 ^{bc}	3.82 ± 0.05 ^{c}	18.35 ± 0.76 ^{ab}	5	654.56 ± 35.04 ^{f}
FB-D	11.18 ± 2.16 ^{b}	5.36 ± 0.05 ^{b}	17.85 ± 1.15 ^{ab}	3	5077.95 ± 129.47 ^{c}
GR-D	23.33 ± 1.16 ^{a}	5.48 ± 0.08 ^{b}	16.43 ± 0.18 ^{bc}	5	501.44 ± 6.73 ^{f}
FM-D	0.76 ± 1.07 ^{d}	0.01 ± 0.01 ^{e}	17.23 ± 1.43 ^{bc}	7	6502.80 ± 199.69 ^{b}
FA-D	2.89 ± 4.08 ^{cd}	5.18 ± 0.15 ^{b}	12.28 ± 1.49 ^{d}	6	2296.25 ± 0.78 ^{e}

BD: Bombay duck (*Harpadon nehereus*); RB: ribbon fish (*Trichiurus iepturus*); WS: white sardine (*Escualosa thoracata*); FB: freshwater barb (*Puntius spp*); GR: Ganges River sprat (*Corica soborna*); FM: fermented barb (*Puntius spp*); FA: fermented anchovies (*Setipnna spp*); D: Dhaka; Different letters (a, b, and c) represent significant differences between fish types via one-way ANOVA.

4.3.4 Circular dichroism (CD)

Secondary structure data is crucial to understanding proteins as the values can reflect the degree of protein degradation and denaturation. Meanwhile, the content of each secondary fraction also affects the functional properties of the protein. As shown in Table 3, the most predominant secondary structure detected in the DFPIs was the random coli (35-40%), followed by β-sheet (20-35%), β-turns (16-25%), and α-helix (6-21%). Sun et al. (2019) reported that in blue round scads (*Decapterus maruadsi*) myosin, the contents of α-helix, β-structures (β-turns + β-sheet) and random coil were 47, 27 and 26%, respectively. The lower α-helix content and higher random coil content of the DFPIs indicate that the protein isolates in current were highly denatured. Tan et al. (2019) reported that solubility of tilapia-soybean protein co-precipitates is negatively correlated with the content of α-helix and positively correlated with β-sheets, which is consistent with the higher solubility of BD-D in our study. Liu et al. (2010) suggested that the β-sheet content is positively related to the gelling ability and gel strength of the protein, which also echoes the relatively low LGC found in BD-D, RF-D and FB-D in current study. However, it should be noted that in addition to the content of secondary structures (α-helix and β-sheet), the amino acid profile and polypeptide composition will also affect the functional properties of each DFPIs (García-Moreno et al., 2016).

The near-UV CD signal originates from the chirality of the side chain environment of amino acid residues, reflecting the strength of the interaction between amino acid residues, thereby revealing changes in the tertiary structure of the protein (Tan et al., 2019). Each aromatic amino acid tends to show a typical peak distribution in the near-UV CD spectrum, such as tyrosine peaks between 275-282 nm, phenylalanine peaks between 255-270 nm, and

tryptophan peaks between 290-305 nm (Kelly et al., 2005). In the current study, except for GR-D, which shows a positive tyrosine peak at 275-282 nm, the ellipticity of the other DFPIs were all close to zero (Figure 2). This suggests that a negligible interaction was detected among aromatic residues in the DFPIs at pH 7.0, which further reveals that the proteins were highly unfolded and had lost most of their native protein structure.

Table 3. Secondary structure fractions of dried fish protein isolate at pH 7.0

Sample ID	α-helix	β-sheet	β-Turns	Random coli	Total
BD-D	9.30 ± 1.27 ^{bc}	30.40 ± 0.99 ^{a}	25.25 ± 8.13 ^{a}	35.00 ± 7.92 ^{b}	99.95 ± 0.07
RF-D	16.25 ± 0.64 ^{ab}	26.40 ± 0.57 ^{ab}	16.75 ± 0.64 ^{b}	40.65 ± 0.92 ^{ab}	100.05 ± 0.21
WS-D	5.75 ± 3.89 ^{c}	35.00 ± 4.53 ^{a}	18.55 ± 0.78 ^{ab}	40.75 ± 1.48 ^{ab}	100.05 ± 0.07
FB-D	6.50 ± 3.68 ^{c}	30.70 ± 2.97 ^{a}	15.95 ± 0.35 ^{b}	46.85 ± 0.35 ^{a}	100.00 ± 0.00
GR-D	8.60 ± 4.81 ^{c}	30.60 ± 4.10 ^{a}	18.05 ± 1.06 ^{ab}	42.70 ± 0.28 ^{ab}	99.95 ± 0.07
FM-D	21.25 ± 0.78 ^{a}	19.80 ± 2.83 ^{b}	19.45 ± 0.21 ^{ab}	39.50 ± 1.70 ^{ab}	100.00 ± 0.14
FA-D	5.75 ± 3.04 ^{c}	35.10 ± 5.52 ^{a}	18.25 ± 0.21 ^{ab}	40.95 ± 2.19 ^{ab}	100.05 ± 0.07

BD: Bombay duck (*Harpadon nehereus*); RB: ribbon fish (*Trichiurus iepturus*); WS: white sardine (*Escualosa thoracata*); FB: freshwater barb (*Puntius spp*); GR: Ganges River sprat (*Corica soborna*); FM: fermented barb (*Puntius spp*); FA: fermented anchovies (*Setipnna spp*); D: Dhaka; Different letters (a, b, and c) represent significant differences between fish types via one-way ANOVA.

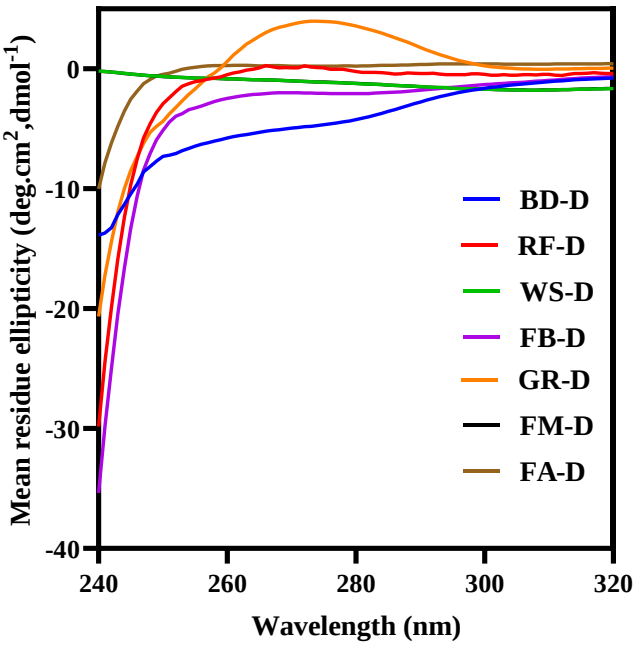

Figure 2. Near_UV circular dichroism spectra at pH 7 of dried fish protein isolates. BD: Bombay duck (*Harpadon nehereus*); RF: ribbon fish (*Trichiurus lepturus*); WS: white sardine (*Escualosa thoracata*); FB: freshwater barb (*Puntius spp*); GR: Ganges River sprat (*Corica soborna*); FM: fermented barb (*Puntius spp*); FA: fermented anchovies (*Setipnna spp*); D: Dhaka.

4.3.5 Heat coagulability (HC)

In general, DFPIs were resistant to heat as the HC of each DFPIs did not surpass 25%, with the highest value of 23.33 ± 1.16 % found in GR-D (Table 2). As a commonly used step in food processing, heat treatment greatly affects the functional performance of proteins in food systems. Under heat treatment, native proteins are first converted into denatured proteins, which then leads to building of insoluble high molecular mass aggregates through intermolecular β-sheet interactions (Nasabi et al., 2017). Therefore, relatively stable thermal stability is desired. The DFPIs in current study have low heat coagulability at pH 7.0, especially FM-D and FA-D, that is, thermal treatment has less effect on the solubility of the protein, which makes it possible to develop the DFPIs as potential ingredients to formulate foods that require thermal processing (Nasabi et al., 2017).

4.3.6 Water (WHC) and oil (OHC) holding capacity

In present study, at pH 7.0, the highest WHC (7.00 ± 0.47 g/g) was found in BD-D, while the lowest was in FM-D with 0.01 ± 0.01g/g (Table 2). Meanwhile, the current findings of WHC of FA-D with 5.18 ± 0.15 g/g, is consistent with the 6 g/g at pH 7.0 WHC reported in a previous study on Argentine anchovy (*Engraulis anchoita*) protein isolates (Rodrigues Freitas et al., 2016). In a previous study (Kumarakuru et al., 2018), the WHC of four fish protein isolates (catfish, *Batrachocephalus mino*; Indian mackerel, *Rastrelliger kanagurta*; ponyfish, *Aurigequula fasciata*; sardine, *Sardinella brachy-soma*) were reported to be between 4.23 ± 0.15 - 4.80 ± 0.05 g/g, which is slightly lower than some of our findings. The WHC of the DFPIs plays a crucial role when it comes to the aspects of mouth feel, flavor retention, as well as texture when developing a new product (Kumarakuru et al., 2018). In this sense, DFPIs in

this study, such as DB-D, FB-D, GR-D, with a higher WHC may favor their uses as ingredients to formulate foods with moist texture.

As for OHC, the highest value was found in BD-D (20.13 ± 0.14 ml/g) while FA-D (12.28 ± 1.49 ml/g) had the lowest value (Table 2). The DFPIs in present study showed a superior OHC values, which are higher than the previous reported values of 3.6, 3.4, and 3.8 ml/g for skin protein hydrolysates produced from grass carp, Nile perch (3.4 ml/g), and Nile tilapia, respectively (Wasswa et al., 2008). The current results are also higher than the 8 ml/g reported for white-mouth croaker (Rodrigues Freitas et al., 2016), and 7.5 ml/g for Argentine anchovy (Rodrigues Freitas et al., 2016) protein isolates. The underlying reason for this may be related to the fact that the fish proteins in the current study may have been oxidized during the drying process, which unfolds the protein structure to expose more hydrophobic groups that can bind to oil molecules (Wasswa et al., 2008). In addition, the dried fish materials have loose and porous physical properties, which could also contribute to better OHC of the DFPIs (Wasswa et al., 2008). OHC is an important attribute in the food industry, especially in the meat and confectionery industries, and is also closely related to product taste because most flavour compounds are soluble only in the oil phase of foods (Wasswa et al., 2008). From the above perspective, BD, and FB with excellent OHC may perform well in the formulation of food products where oil retention is a great contributor to quality and consumer acceptance.

4.3.7 Least gelation concentration (LGC)

We found that the gelling ability or the LGC of the DFPIs was different according to the dried fish type, as the highest LGC was for FM-D with 7% and lowest was found in BD-D, RF-D, and FB-D at 3% (Table 2). The lower the value, the stronger the gelling ability of the

protein. In general, the gelling ability of the DFPIs is better than that of the isolates extracted from legumes, as the reported LGCs of soybean, pea, faba bean, and lentil, ranged between 12-15% (Ma et al., 2022), which are higher than the values found in present study. The current findings of the LGC of the DFPIs are in agreement with the finding of 6% LGC in alkali extracted saithe (*Pollachius virens*) isolates (Shaviklo et al., 2012). The LGC of DFPIs is comprehensively affected by different factors, including the degree of denaturation of myofibrillar proteins and the ratio of myofibrillar/sarcoplasmic proteins (Shaviklo et al., 2012). In addition, factors such as molecular weight distribution, the effective volume fraction, and the chemical and physical interactions formed by proteins during the thermal treatment will also affect the LGC of DFPIs (Ma et al., 2022). The high gelling ability of the DFPIs suggests that they may be used to make fish protein gels like surimis that are important ingredients in the formulation of imitation shellfish products.

4.3.8 Emulsion formation and stability

In the food industry, protein isolates are widely used as emulsifiers in multiple food systems, such as beverages, sausages, salad dressings, cakes, and soups, because of their ability to reduce the interfacial tension between water and the lipid phase and form a protective coating that prevents the oil droplets from coalescence (Ma et al., 2022). Droplet size reduction is the key target when it comes to emulsion formation ability. In present study, the DFPIs at all concentrations showed a relatively good emulsion forming ability, as the maximum droplet size at pH 7.0 was found in RF-D (~4.5 μm) while the minimum was as small as ~2 μm for the emulsion formed with 20 mg/ml BD-D. The emulsion oil droplet sizes obtained in current study with values of about 3.6 μm are consistent with a previously reported oil droplet size of 20

mg/ml hydrated raw sardines' protein (3.672 ± 0.179 μm), at pH 2.0 (García-Moreno et al., 2016). However, at pH 2.0, fish proteins are more soluble than at pH 7.0, and consequently easier to adsorb at the oil-water interface, which may also explain the smaller droplet size found in BD-D (highest solubility among all DFPIs). Within the same pH and protein concentration, an even smaller oil droplet size of DFPIs emulsion can be expected. Ma et al. (2022) reported that in addition to solubility, protein concentration also significantly affected the droplet size, as a significant decrease of droplet size of emulsions formed with soy protein isolate was obtained at the 10 mg/ml concentration (2.5 μm) when compared to the 19 μm for 1 mg/ml. A less severe but still statistically significant decrease of droplet size was detected in present study, which may suggest that the DFPIs concentrations used in current were sufficient to form good emulsions.

Figure 3 also included the information of emulsion stability (ES), which generally indicates that the BD-D had the best overall ES as reflected in the 100% value at 10 and 15 mg/ml. The underlying reason for this may be related to the higher solubility of the BD-D. In the current study, our findings of ES values close to 70% at 10 mg/ml of protein content, are consistent with a previously reported ES of 71.3% (Shaviklo et al., 2012), which was observed for saithe (*Pollachius virens*) emulsions. Apart from the solubility, protein concentration may be another crucial reason to maintain a stable emulsion, as detected in RF-D, WS-D, GR-D and FA-D, with increasing the protein concentration the value of the ES increased significantly. The results are aligned with the findings of Rajasekaran et al. (2022) who applied ultrasonic treatment to form a fish protein-coated shrimp oil emulsions and the ES increased from 61.54% ± 84.75% with increasing concentration of 15-45 mg/ml after a 15-days of storage. This can be

explained by the presence of more protein molecules in the oil-water interface to interact together and form strong membranes around the oil droplets, which provides stability against oil droplet coalescence.

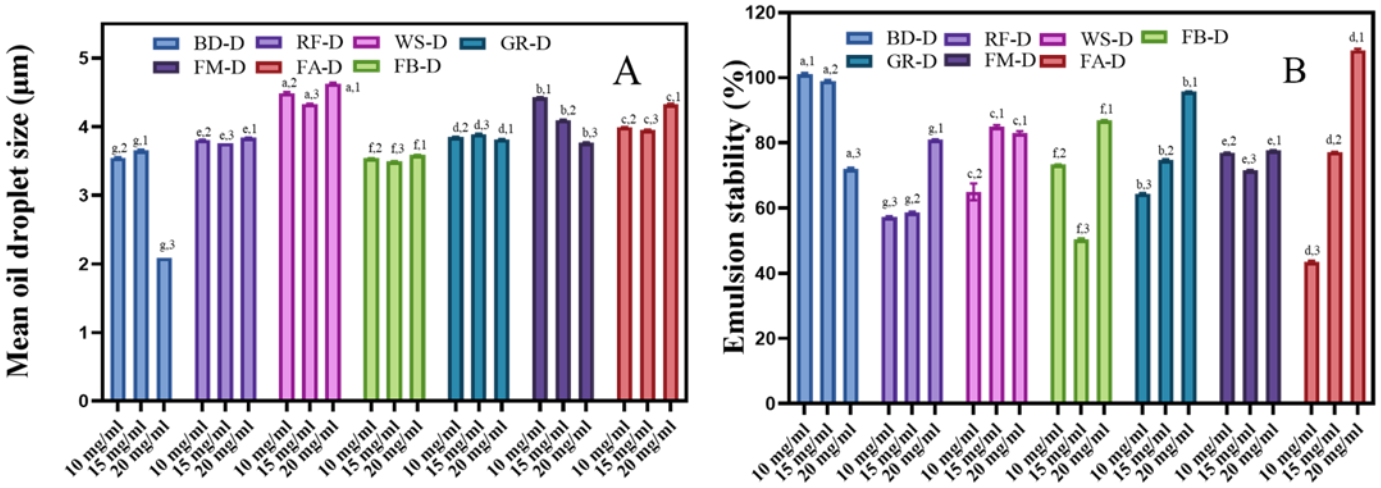

Figure 3. Emulsion data of dried fish protein isolates (DFPIs) at pH 7.0; A: emulsion forming ability; B: emulsion stability. BD: Bombay duck (*Harpadon nehereus*); RF: ribbon fish (*Trichiurus lepturus*); WS: white sardine (*Escualosa thoracata*); FB: freshwater barb (*Puntius spp*); GR: Ganges River sprat (*Corica soborna*); FM: fermented barb (*Puntius spp*); FA: fermented anchovies (*Setipnna spp*); D: Dhaka. Different letters (a, b, and c) represent significant differences between fish types via two-way ANOVA. Different numbers (1, 2, and 3) represent significant differences within the same fish type and different protein concentration (mg/ml) via one-way ANOVA.

4.4 Conclusion

This study extracted seven DFPIs and explored their functional properties at pH 7. It was found that the high-salt extraction environment (inherited in the DFs) of DFPIs moved its previously reported IP of pH 5.5 to a more acidic pH 4.5 and influenced the ratio of myofibrillar protein and sarcoplasmic protein in the isolates to differ from that of fresh fish protein isolates. At pH 7.0, the DFPIs were not highly sensitive to heat, exhibiting low heat coagulability (generally less than 20%), which give DFPIs the potential to be used to formulate thermally processed foods. The high OHC (>11 mg/ml), excellent gelling ability (LGC ≤ 8%), and relatively excellent emulsification ability (oil droplets size ≤ 5 μm; a generally emulsion

stability >70%) suggest potential use of the isolates in the formulation of salad dressings and mayonnaise in addition to meat products such as hamburgers, hot dogs, and sausages. The high solubility of some of the isolates means that they could find useful applications in beverage fortifications. Results from CD (reduced α-helix structure and elevated β-sheet and random coli structure compared to FPIs), SDS-PAGE (scrambled polypeptide bands), and surface hydrophobicity (higher surface hydrophobicity) showed that DFPIs are highly denatured, unfolded, and degraded protein mixtures, which is consistent with their performance in the functional tests.

REFERENCE

Adeleke, R. O., & Odedeji, J. O. (2010). Acceptability studies on bread fortified with tilapia fish flour. *Pakistan Journal of Nutrition*, *9*(6), 531-534.

Aluko, R. E., & Yada, R. Y. (1997). Some Physicochemical and Functional Properties of Cowpea (*Vigna Unguiculata*) Isoelectric Protein Isolate as a Function of PH and Salt Concentration. *International Journal of Food Sciences and Nutrition*, *48*(1), 31–39. https://doi.org/10.3109/09637489709006961

Álvarez, C., Lélu, P., Lynch, S. A., & Tiwari, B. K. (2018). Optimised protein recovery from mackerel whole fish by using sequential acid/alkaline isoelectric solubilization precipitation (ISP) extraction assisted by ultrasound. *LWT*, *88*, 210–216. https://doi.org/10.1016/j.lwt.2017.09.045

Banna, Md. H. Al, Al Zaber, A., Rahman, N., Siddique, M. A. M., Siddique, M. A. B., Hagan, J. E., Rifat, M. A., Nsiah-Asamoah, C. N. A., Seidu, A.-A., Ahinkorah, B. O., & Khan, M. S. I. (2022). Nutritional Value of Dry Fish in Bangladesh and Its Potential Contribution to Addressing Malnutrition: A Narrative Review. *Fishes*, *7*(5), 240. https://doi.org/10.3390/fishes7050240

Belton, B., Johnson, D. S., Thrift, E., Olsen, J., Hossain, M. A. R., & Thilsted, S. H. (2022). Dried fish at the intersection of food science, economy, and culture: A global survey. *Fish and Fisheries*, *23*(4), 941–962. https://doi.org/10.1111/faf.12664

Bhowmik, S., Zakaria, M. A., Sarwar, Md. S., Shofi, S. B., Syduzzaman, Akter, F., Islam, Md. M., & Mamun, A.-A. (2022). Development and nutritional index of ready to use fish products (RUFPs) from small fish species: Future superfoods for consumers. *Applied Food*

Research, *2*(1), 100111. https://doi.org/10.1016/j.afres.2022.100111

Chao, D., Jung, S., & Aluko, R. E. (2018). Physicochemical and functional properties of high pressure-treated isolated pea protein. *Innovative Food Science & Emerging Technologies*, *45*, 179–185. https://doi.org/10.1016/j.ifset.2017.10.014

Chen, Y.-C., & Jaczynski, J. (2007). Protein Recovery from Rainbow Trout (*Oncorhynchus mykiss*) Processing Byproducts via Isoelectric Solubilization/Precipitation and Its Gelation Properties As Affected by Functional Additives. *Journal of Agricultural and Food Chemistry*, *55*(22), 9079–9088. https://doi.org/10.1021/jf071992w

Chung, Y.-C., Ho, M.-L., Chyan, F.-L., & Jiang, S.-T. (2000). Utilization of freeze-dried mackerel (Scomber australasicus) muscle proteins as a binder in restructured meat. *Fisheries Science*, *66*(1), 130–135. https://doi.org/10.1046/j.1444-2906.2000.00019.x

Ding, J., Zhao, X., Li, X., & Huang, Q. (2022). Effects of different recovered sarcoplasmic proteins on the gel performance, water distribution and network structure of silver carp surimi. *Food Hydrocolloids*, *131*, 107835. https://doi.org/10.1016/j.foodhyd.2022.107835

Feng, X., Yu, X., Yang, Y., & Tang, X. (2023). Improving the freeze-thaw stability of fish myofibrils and myofibrillar protein gels: Current methods and future perspectives. *Food Hydrocolloids*, *144*, 109041. https://doi.org/10.1016/j.foodhyd.2023.109041

García-Moreno, P. J., Guadix, A., Guadix, E. M., & Jacobsen, C. (2016). Physical and oxidative stability of fish oil-in-water emulsions stabilized with fish protein hydrolysates. *Food Chemistry*, *203*, 124–135. https://doi.org/10.1016/j.foodchem.2016.02.073

Gehring, C. K., Gigliotti, J. C., Moritz, J. S., Tou, J. C., & Jaczynski, J. (2011). Functional and nutritional characteristics of proteins and lipids recovered by isoelectric processing of fish

by-products and low-value fish: A review. *Food Chemistry*, *124*(2), 422–431. https://doi.org/10.1016/j.foodchem.2010.06.078

Hamdani, Rizal, T. A., & Muhammad, Z. (2018). Fabrication and testing of hybrid solar-biomass dryer for drying fish. *Case Studies in Thermal Engineering*, *12*, 489–496. https://doi.org/10.1016/j.csite.2018.06.008

Hashimoto, K., Watabe, S., KoNo, M., & Shiro, K. (1979). Muscle protein composition of sardine and mackerel. *Bulletin of the Japanese Society of Scientific Fisheries*, *45*(11), 1435–1441.

Haskard, C. A., & Li-Chan, E. C. Y. (1998). Hydrophobicity of Bovine Serum Albumin and Ovalbumin Determined Using Uncharged (PRODAN) and Anionic (ANS $^{-}$) Fluorescent Probes. *Journal of Agricultural and Food Chemistry*, *46*(7), 2671–2677. https://doi.org/10.1021/jf970876y

Hussain, I., Akhtar, N., & Hussain, S. (2007). Evaluation of weaning food khitchri incorporated with different levels of fish protein concentrate. . . *Animal Plant Sciences*, *17*(1–2), 12–17.

Ibrahim, S. M. (2009). Evaluation of production and quality of salt-biscuits supplemented with fish protein concentrate. *World Journal of Dairy and Food Sciences*, *4*(1), 28–31.

Kelly, S. M., Jess, T. J., & Price, N. C. (2005). How to study proteins by circular dichroism. *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics*, *1751*(2), 119–139. https://doi.org/10.1016/j.bbapap.2005.06.005

Kobayashi, Y., & Park, J. W. (2018). Optimal blending of differently refined fish proteins based on their functional properties. *Journal of Food Processing and Preservation*, *42*(1), e13346. https://doi.org/10.1111/jfpp.13346

Kumarakuru, K., Reddy, C. K., & Haripriya, S. (2018). Physicochemical, morphological and functional properties of protein isolates obtained from four fish species. *Journal of Food Science and Technology*, *55*(12), 4928–4936. https://doi.org/10.1007/s13197-018-3427-0

Laemmli, U. K. (1970). Cleavage of Structural Proteins during the Assembly of the Head of Bacteriophage T4. *Nature*, *227*(5259), 680–685. https://doi.org/10.1038/227680a0

Liu, R., Zhao, S., Liu, Y., Yang, H., Xiong, S., Xie, B., & Qin, L. (2010). Effect of pH on the gel properties and secondary structure of fish myosin. *Food Chemistry*, *121*(1), 196–202. https://doi.org/10.1016/j.foodchem.2009.12.030

Ma, K. K., Grossmann, L., Nolden, A. A., McClements, D. J., & Kinchla, A. J. (2022). Functional and physical properties of commercial pulse proteins compared to soy derived protein. *Future Foods*, *6*, 100155. https://doi.org/10.1016/j.fufo.2022.100155

Malomo, S. A., He, R., & Aluko, R. E. (2014). Structural and Functional Properties of Hemp Seed Protein Products. *Journal of Food Science*, *79*(8). https://doi.org/10.1111/1750-3841.12537

Markwell, M. A. K., Haas, S. M., Bieber, L. L., & Tolbert, N. E. (1978). A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. *Analytical Biochemistry*, *87*(1), 206–210. https://doi.org/10.1016/0003-2697(78)90586-9

Mohd Khairi, I. N. B., Huda, N., Wan Abdullah, W. N., & Al-Kharki, A. F. M. (2014). Protein quality of fish fermented product: budu and rusip. *Asia Pacific Journal of Sustainable Agriculture, Food and Energy*, *2*(2), 17-22.

Nagwekar, N., Tidke, V., & Thorat, B. N. (2017). Microbial and biochemical analysis of dried fish and comparative study using different drying methods. *Drying Technology*, *35*(12),

1481–1491. https://doi.org/10.1080/07373937.2016.1256889

Nasabi, M., Labbafi, M., Mousavi, M. E., & Madadlou, A. (2017). Effect of salts and nonionic surfactants on thermal characteristics of egg white proteins. *International Journal of Biological Macromolecules*, *102*, 970–976. https://doi.org/10.1016/j.ijbiomac.2017.04.102

Ochiai, Y., & Ozawa, H. (2020). Biochemical and physicochemical characteristics of the major muscle proteins from fish and shellfish. *Fisheries Science*, *86*(5), 729–740. https://doi.org/10.1007/s12562-020-01444-y

Osemwota, E. C., Alashi, A. M., & Aluko, R. E. (2021). Comparative Study of the Structural and Functional Properties of Membrane-Isolated and Isoelectric pH Precipitated Green Lentil Seed Protein Isolates. *Membranes*, *11*(9), 694. https://doi.org/10.3390/membranes11090694

Pires, C., Costa, S., Batista, A. P., Nunes, M. C., Raymundo, A., & Batista, I. (2012). Properties of protein powder prepared from Cape hake by-products. *Journal of Food Engineering*, *108*(2), 268–275. https://doi.org/10.1016/j.jfoodeng.2011.08.020

Poljanec, I., Marušić Radovčić, N., Petričević, S., Karolyi, D., Listeš, E., & Medić, H. (2021). Proteolysis and protein oxidation throughout the smoked dry-cured ham process. *Food Chemistry*, *362*, 130207. https://doi.org/10.1016/j.foodchem.2021.130207

Rahman, M. A., Saifullah, M., & Islam, M. N. (2012). Fish powder in instant fish soup mix. *Journal of the Bangladesh Agricultural University*, *10*(452-2016–35550), 145–148.

Raikos, V., Neacsu, M., Russell, W., & Duthie, G. (2014). Comparative study of the functional properties of lupin, green pea, fava bean, hemp, and buckwheat flours as affected by

<scp>pH</scp>. *Food Science & Nutrition*, *2*(6), 802–810. https://doi.org/10.1002/fsn3.143

Rajasekaran, B., Singh, A., Zhang, B., Hong, H., & Benjakul, S. (2022). Changes in Emulsifying and Physical Properties of Shrimp Oil/Soybean Oil-in-Water Emulsion Stabilized by Fish Myofibrillar Protein during the Storage. *European Journal of Lipid Science and Technology*, *124*(10). https://doi.org/10.1002/ejlt.202200068

Ramírez, J. A., Díaz-Sobac, R., Morales, O. G., & Vázquez, M. (1999). Evaluation of freeze-dried surimi from tilapia and fat sleeper as emulsifiers Evaluación de surimi liofilizado de tilapia y dormidor como emulsificantes evaluación de surimi liofilizado de tilapia e dormidor como emulsificantes. *Ciencia y Tecnologia Alimentaria*, *2*(4), 210–214. https://doi.org/10.1080/11358129909487604

Reza Shaviklo, A. (2012). Developing value-added products from lantern fish. *Infofish International*, *2*(42).

Rodrigues Freitas, I., Cortez-Vega, W. R., & Prentice, C. (2016). Physicochemical and Functional Properties of Protein Recovered from Fish Waste. *Journal of Aquatic Food Product Technology*, *25*(7), 1034–1044. https://doi.org/10.1080/10498850.2015.1008714

Sathivel, S., Bechtel, P. J., Babbitt, J. K., Prinyawiwatkul, W., & Patterson, M. (2005). Functional, Nutritional, and Rheological Properties of Protein Powders from Arrowtooth Flounder and their Application in Mayonnaise. *Journal of Food Science*, *70*(2), E57–E63. https://doi.org/10.1111/j.1365-2621.2005.tb07091.x

Shamsuzzaman, Md. M., Hoque Mozumder, M. M., Mitu, S. J., Ahamad, A. F., & Bhyuian, Md. S. (2020). The economic contribution of fish and fish trade in Bangladesh. *Aquaculture*

and Fisheries, *5*(4), 174–181. https://doi.org/10.1016/j.aaf.2020.01.001

Shaviklo, A. R. (2015). Development of fish protein powder as an ingredient for food applications: a review. *Journal of Food Science and Technology*, *52*(2), 648–661. https://doi.org/10.1007/s13197-013-1042-7

Shaviklo, A. R., & Etemadian, Y. (2019). Overcoming current challenges in commercial applications of fish protein isolates in food and feed systems: a review. *Journal of Food Science and Technology*, *56*(11), 4775–4784. https://doi.org/10.1007/s13197-019-03966-5

Shaviklo, G. R., Olafsdottir, A., Sveinsdottir, K., Thorkelsson, G., & Rafipour, F. (2011). Quality characteristics and consumer acceptance of a high fish protein puffed corn-fish snack. *Journal of Food Science and Technology*, *48*(6), 668–676. https://doi.org/10.1007/s13197-010-0191-1

Shaviklo, G. R., Thorkelsson, G., Arason, S., & Sveinsdottir, K. (2012). Characteristics of freeze-dried fish protein isolated from saithe (Pollachius virens). *Journal of Food Science and Technology*, *49*(3), 309–318. https://doi.org/10.1007/s13197-011-0285-4

Shaviklo, G. R., Thorkelsson, G., Sveinsdottir, K., & Pourreza, F. (2013). Studies on processing, consumer survey and storage stability of a ready-to-reconstitute fish cutlet mix. *Journal of Food Science and Technology*, *50*(5), 900–908. https://doi.org/10.1007/s13197-011-0417-x

Shaviklo, G. R., Thorkelsson, G., Sveinsdottir, K., & Rafipour, F. (2011). Chemical properties and sensory quality of ice cream fortified with fish protein. *Journal of the Science of Food and Agriculture*, *91*(7), 1199–1204. https://doi.org/10.1002/jsfa.4299

Sun, L.-C., Lin, Y.-C., Liu, W.-F., Qiu, X.-J., Cao, K.-Y., Liu, G.-M., & Cao, M.-J. (2019). Effect of pH shifting on conformation and gelation properties of myosin from skeletal muscle of blue round scads(Decapterus maruadsi). *Food Hydrocolloids*, *93*, 137–145. https://doi.org/10.1016/j.foodhyd.2019.02.026

Surasani, V. K. R., Raju, C., Sofi, F. R., & Shafiq, U. (2022). Utilization of protein isolates from rohu (*Labeo rohita*) processing waste through incorporation into fish sausages; quality evaluation of the resultant paste and end product. *Journal of the Science of Food and Agriculture*, *102*(3), 1263–1270. https://doi.org/10.1002/jsfa.11464

Tadpitchayangkoon, P., Park, J. W., & Yongsawatdigul, J. (2010). Conformational changes and dynamic rheological properties of fish sarcoplasmic proteins treated at various pHs. *Food Chemistry*, *121*(4), 1046–1052. https://doi.org/10.1016/j.foodchem.2010.01.046

Tan, L., Hong, P., Yang, P., Zhou, C., Xiao, D., & Zhong, T. (2019). Correlation Between the Water Solubility and Secondary Structure of Tilapia-Soybean Protein Co-Precipitates. *Molecules*, *24*(23), 4337. https://doi.org/10.3390/molecules24234337

Tan, Y., & Chang, S. K. (2021). Protein extraction <scp>pH</scp> and cross-linking affect physicochemical and textural properties of protein gels made from channel catfish by-products. *Journal of the Science of Food and Agriculture*, *101*(11), 4799–4807. https://doi.org/10.1002/jsfa.11126

Wang, P. A., Vang, B., Pedersen, A. M., Martinez, I., & Olsen, R. L. (2011). Post-mortem degradation of myosin heavy chain in intact fish muscle: Effects of pH and enzyme inhibitors. *Food Chemistry*, *124*(3), 1090–1095. https://doi.org/10.1016/j.foodchem.2010.07.093

Wang, W., Xia, W., Gao, P., Xu, Y., & Jiang, Q. (2017). Proteolysis during fermentation of Suanyu as a traditional fermented fish product of China. *International Journal of Food Properties*, *20*(sup1), S166–S176. https://doi.org/10.1080/10942912.2017.1293089

Wasswa, J., Tang, J., & Gu, X. (2008). Functional Properties of Grass Carp (*Ctenopharyngodon Idella*), Nile Perch (*Lates Niloticus*) and Nile Tilapia (*Oreochromis Niloticus*) Skin Hydrolysates. *International Journal of Food Properties*, *11*(2), 339–350. https://doi.org/10.1080/10942910701381188

Whitmore, L., & Wallace, B. A. (2004). DICHROWEB, an online server for protein secondary structure analyses from circular dichroism spectroscopic data. *Nucleic Acids Research*, *32*(Web Server), W668–W673. https://doi.org/10.1093/nar/gkh371

Xiong, Y., Li, Q., Miao, S., Zhang, Y., Zheng, B., & Zhang, L. (2019). Effect of ultrasound on physicochemical properties of emulsion stabilized by fish myofibrillar protein and xanthan gum. *Innovative Food Science & Emerging Technologies*, *54*, 225–234. https://doi.org/10.1016/j.ifset.2019.04.013

Zhong, S., Liu, S., Cao, J., Chen, S., Wang, W., & Qin, X. (2016). Fish Protein Isolates Recovered from Silver Carp (*Hypophthalmichthys molitrix*) By-Products Using Alkaline pH Solubilization and Precipitation. *Journal of Aquatic Food Product Technology*, *25*(3), 400–413. https://doi.org/10.1080/10498850.2013.865282

CHAPTER FIVE

GENERAL SUMMARY AND CONCLUSIONS

The macro- and micro-nutrient contents of seven Bangladeshi DFs collected from four cities were explored in this study. Meanwhile the functional properties of Dhaka DFPIs were also determined. Two medium/large fish, three indigenous small fish, and two fermented small fishes were included. With regards to nutritional properties, two indigenous small fish species, WS and GR, can provide significantly more minerals (calcium, potassium, manganese, iron, copper, and zinc), n-3 unsaturated fatty acids (EPA, DHA), essential amino acids, and vitamin B_{12} to the vulnerable groups' daily dietary requirements than other DFs. FB (another indigenous small fish) can also meet daily nutritional needs in terms of multiple nutrients, but compared with the previously mentioned WS and GR, its higher sodium content, lower protein content and quality, lower vitamin B_{12} content and relatively inferior fatty acid composition may have some negative effects on consumers' health or may provide relatively lower nutritional benefits. Medium/large DFs perform moderately in terms of daily nutritional contribution, that is, they contain moderate levels of various nutrients. However, high cholesterol and high SFA contents may cause related health risks to consumers who consume such DFs for a long time. Fermented small fish contains large amounts of ash, especially sodium, calcium, manganese, and chromium, which need to be treated with caution as excessive intake may cause consequent harmful effects. In addition, lipid degradation and oxidation caused by fermentation may lead to poor fatty acid distribution (unsaturated fatty acids are oxidized into saturated fatty acids). However, the fermentation process degraded the original structure of the protein, which resulted in an improved protein digestibility. In addition, the significantly lower cholesterol

content and higher FAA content found in fermented DFs may be beneficial to consumer acceptance in terms of health and flavor. It should be noted that all DF used in this study, except WS and FA, were found to have excessive levels of heavy metals, which may not only be due to the concentration effect of drying but also may be from water pollution and irregular transportation and storage.

During the preparation of DFPIs, we found that the high-salt extraction environment (inherited from DF) shifted its previously reported IP from pH 5.5 to a more acidic pH 4.5 and most likely affected the myofibrillar proteins/myofibrils ratio in the isolates. Therefore, the protein isolates behaved differently from fresh fish protein isolates in terms of composition and functional properties. DFPIs exhibited a low heat sensitivity (overall less than 20% thermal coagulation) at pH 7.0, which may be related to their irreversible denaturation during heat processing. However, the DFPIs exhibited a high OHC (>11 mg/ml), excellent gelling ability (LGC ≤ 8%), relatively excellent emulsification, and good emulsion stability (oil droplet size ≤ 5 μm; general emulsification Stability >70%), which make DFPIs promising for formulating heat-processed foods. DFPIs showed a high surface hydrophobicity, which could have contributed to the strong emulsifying capacity. Data from CD analysis revealed that compared with FPI, DFPIs had reduced α-helical structure, increased β-sheet and random coil structures. SDS-PAGE results showed that DFPIs had irregular and broad polypeptide composition bands. The above description confirms that DFPIs are highly denatured, unfolded and degraded protein mixtures.

Taking into account the heavy metal content and the distribution of various nutrients in DFs, the small fishes, especially WS, may become important tools to alleviate malnutrition

among vulnerable groups and enhance food security. However, the health risks associated with consumption of DFs do not only come from heavy metals. To ensure food safety, the pollutants that need to be monitored should not be limited to heavy metals, but also include chemical residues (pesticides), foreign matter (sand), microorganisms (pathogens and fungi), and microplastics (commonly found in DFs). With regard to the performance of DFPIs in functional tests, it may be beneficial to expand their potential in developing functional food additives, such as emulsifiers, foaming agents, and gelling agents. Due to the high-level degradation, DFPIs may also qualify to be developed into health-beneficial short peptides products, thereby increasing the possibility of added value.