Sub-Project ID -157

Program Based Research Grant (PBRG)

Sub-project Completion Report

on

Development of Fish-based Food Products and Extension of Shelf-life to Enhance Nutritional Security

Sub-project Duration

15 October 2019 to 29 December 2022

Coordinating Organization

Fisheries Division
Bangladesh Agricultural Research Council
Farmgate, Dhaka-1215

Project Implementation Unit

National Agricultural Technology Program-Phase II Project

Bangladesh Agricultural Research Council
Farmgate, Dhaka-1215

December 2022

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Implementing Organization

Department of Fisheries & Marine Science
Noakhali Science and Technology University
Noakhali-3814

Department of Fisheries
University of Rajshahi
Rajshahi-6205

Project Implementation Unit

National Agricultural Technology Program-Phase II Project
Bangladesh Agricultural Research Council
Farmgate, Dhaka-1215

December 2022

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National Agricultural Technology Program-Phase II Project (NATP-2)
Bangladesh Agricultural Research Council (BARC)
New Airport Road, Farmgate, Dhaka – 1215
Bangladesh

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67 Noya Paltan, Dhaka-1000
alam.nurey13@gmail.com

Abbreviation and Acronyms

°C	Degree Celsius	LF	Local Facilitator
µg	Microgram	LM	Linear Model
µmol	Micromole	LMIC's	Low Medium-Income Countries
AA	Amino Acids	MA	Modified Atmosphere
ANCOVA	Analysis of Covariance	MAP	Modified Atmosphere Packaging
ANOVA	Analysis of Variance	MDA	Malondialdehyde
AOAC	Association of Official Analytical Chemists	mg	Milligram
APC	Aerobic Plate Count	mL	Milliliter
BARC	Bangladesh Agricultural Research Council	MUAC	Mid-Upper Arm Circumference
BCR	Benefit-Cost Ratio	MUFA	Monounsaturated Fatty Acids
BDT	Bangladeshi Taka	NAS	Nutritional Analytical Service
BHT	Butylated Hydroxytoluene	NATP-2	National Agricultural Technology Program phase-2
BMI	Body Mass Index	NGO	Non-Governmental Organization
BO	Better-off	NIH	National Institute of Health
CFU	Colony Forming Unit	NSTU	Noakhali Science and Technology University
CHD	Coronary Heart Disease/ Cardiac Heart Disease	OECD	Organization of Economic Co-operation and Development
Co-PI	Co-Principal Investigator	PBRG	Program Based Research Grant
CRP	C-reactive Protein	PI	Principal Investigator
CVD	Cardiovascular Disease	PIU	Project Implementation Unit
DHA	Docosahexaenoic Acids [(22:6(n-3)]	PLW	Pregnant and Lactating Women
DMRT	Duncan Multiple Range Test	PPM	Part Per Million
DoF	Department of Fisheries	PPS	Peptone Physiological Saline
DPA	Docosapentaenoic Acid [22:5(n-3)]	PPT	Parts Per Thousands
EAA	Essential Amino Acid	PRA	Participatory Rural Appraisal
EPA	Eicosapentaenoic Acids [20:5(n-3)]	PUFA	Polyunsaturated Fatty Acids
FAME	Fatty Acid Methyl Esters	PV	Peroxide Value
FAO	Food and Agricultural Organization	RBC	Red Blood Cell
FFA	Free Fatty Acid	RCT	Randomized controlled Trial
FIQC	Fish Inspection and Quality Control	RDA	Recommended Daily Allowance
FPVP	Fish Powder Vacuum Package	RNI	Recommended Nutritional Intake
FPZP	Fish Powder Zipper Package	RTC	Ready-to-cook
FVC	Food Value Chain	RTE	Ready-to-eat
FW	Freshwater	RU	Rajshahi University
GC	Gas Chromatography	RUFP	Ready to Use Fish Products

GDP	Gross Domestic Production	SD	Standard Deviation
GLC	Gas-Liquid Chromatography	SDG	Sustainable Development Goal
GPS	Global Positioning System	SFA	Saturated Fatty Acids
Ha	Hectare	SPSS	Statistical Package for Social Sciences
HBS	Household Budget Survey	SWOT	Strengths, Weaknesses, Opportunities, and Threats
HH	Household	TAB	Tablet (electronic device)
HIV	Human Immunodeficiency Virus	TBARS	Thiobarbituric Acid Reactive Substance
HPLC	High-Performance Liquid Chromatograph	TLC	Thin-Layer Chromatography
HUFA	Highly Unsaturated Fatty Acids	TN	Total Nitrogen
ICDDRB	International Centre for Diarrheal Disease Research, Bangladesh	TOC	Total Organic Carbon
ICP-AES	Inductively Coupled Plasma–Atomic Emission Spectrometry	TP	Total Phosphate
ICP-MS	Inductive Coupled Plasma-Mass Spectrometry	TSP	Triple Super Phosphate
IFAD	International Fund for Agricultural Development	TVBN	Total Volatile Base Nitrogen
IHFA	Intra-Household Food Allocation	TVC	Total Viable Count
INFS	Institute of Nutrition and Fitness Sciences	UK	United Kingdom
Kg	Kilogram	USDA	United States Department of Agriculture
KIs	Key Informants	VP	Vacuum Packaging
LC-PUFA	Long-Chain Polyunsaturated Fatty Acids	WB	World Bank

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Executive Summary

Despite the rapid expansion of aquaculture production, as well as, progression in many other social, economic, and health indicators due to the direct or indirect contribution of the sub-sector, still, at least 40% of Bangladeshi have been sufferings from various malnutrition burdens. Efficient utilization of unutilized and under-utilized fish catch and reduction of post-harvest fish loss can remarkably contribute thereby providing a continuous supply of a higher amount of fish protein along with many essential micronutrients for human consumption. Ready-to-use food products (RUFPs) like Fish powder, Fish balls, Fish crackers, Fish sausages, Fish marinades, etc. could potentially provide an option to mitigate malnutrition challenges at household levels in rural and urban areas of the country. Thus, to develop fish-based value-added RUFPs and to ensure the food and nutritional security of the vulnerable population of the country, the research sub-project entitled "Development of fish-based food products and extension of shelf life to enhance nutritional security" was executed jointly by the Department of Fisheries and Marine Science, Noakhali Science and Technology University, Noakhali-3814 and Department of Fisheries, University of Rajshahi, Rajshahi-6205 with the funding support of NATP 2, PIU, BARC during October'2019 to October'2022.

Under the set objectives of component 1 (NSTU), the Fish powder was developed from five available, low-priced, and nutritious fish species (Punti, *Puntius ticto;* Kachki, *Corica soborna;* Chapila, *Gudusia chapra;* Sardine, *Sardinella fimbriata* and Anchovy, *Coilia dussumieri*). From five fish species, 13 different types of powder were developed where only Kachki was used for preparing whole Fish powder and the remaining species were used as whole, gutted, and muscle portions. The nutrient content including macronutrients (protein, lipid, ash, and moisture energy), fatty acids profile, amino acids, and micronutrients were analyzed and whole fish powders were provide with greater ranges of micronutrients. Among all Chapila whole fish powder was selected for the intervention. Three sites (Lakshmipur, Noakhali, and Khagrachari with two arms from each site were selected for the study. Every intervening household with at least one unmarried adolescent girl (10-19 years) got Fish powder and token money to cook the desired recipe on a weekly basis. Anthropometric (BMI, MUAC) were evaluated by baseline and end-line field surveys, and blood samples from eligible adolescent girls were collected for biochemical analysis (Fe, Ca, vitamin A, vitamin B12, ferritin, CRP). In addition, socioeconomic information and dietary information were gathered at the household level using the 24-hour recall approach. After the intervention of 16 weeks, Fish powder put a significant impact on energy and nutrient supply in adolescent girls and was also manifested in the nutritional outcomes. According to the findings, vitamin B_{12} was increased in the blood serum of adolescent girls living in the intervention sites compared to baseline and control sites. The water iron level in the tube wells was significantly different among the three agro-ecologies. However, water iron has a minimum impact on the serum Fe and Hb levels of adolescent girls. An in-vivo study on Swiss albino mice that were fed with different doses of Fish powder for 28 days indicated that 100 g/BW doses have resulted in the upliftment of key biomarkers including Fe and Vitamin B_{12}. Another separate study on consumer preferences in urban settings indicated that more than two-thirds considered Fish Powder as a well-accepted food item in their basket. The study finally considered the shelf life of Fish powder and it is expected to last for one year at room temperature. These all above experiments have provided a holistic scenario on Fish powder selection to RNI for individuals. A Ready-to-use food-based product (Fish powder) in food baskets and people's plates is expected to reach through the involvement of industry would be a great deal for sustainable food systems of the planet.

Component 2 (RU) of the sub-project under its set objectives, at first developed seven Ready-to-cook (RTC) and two Ready-to-eat (RTE) products from freshwater and marine fishes followed by the assessment of the quality of the products covering proximate composition analysis, texture, color, and sensory analysis. In 2nd stage, four types of packaging namely, (a) not sealed pack as control; (b) MAP 1 (50%CO_2 & 50%N_2) as treatment-1; (c) MAP 2 (40% CO_2, 30% N_2 & 30% O_2) at treatment-2 and (d) MAP 3 (50% CO_2 & 50% O_2) as treatment-3 were applied for RTC products under modified atmosphere packaging (MAP) using a tray sealer (Oceania mini, Italian pack, Italy). In contrast, three types of packaging, namely, (a) air pack as control; (b) MAP 1 (100% N_2) as treatment-1 and (c) MAP 2 (50% CO_2 & 50%N_2) at treatment-2, were done for RTE fish products in a plastic pouch by the same way using pouch packaging unit (C100, Multivac, Germany). The shelf life of those products was then evaluated by measuring pH, FFA, TVBN, TBARS, and APC under refrigerated (4˚C) conditions at 3-10 days and atmospheric storage at a monthly interval, respectively. In the third stage, consumers' acceptability and willingness to buy the packaged RTC and RTE fish products were investigated by survey method utilizing a structured questionnaire in three outlets such as Amana big Bazar, Rajshahi; Rajshahi University souvenir shop, Rajshahi; BARC canteen, Farmgate, Dhaka. All the surveyed data were subjected to descriptive analyses, and laboratory data were subjected to one-way ANOVA with the application of the Tukey test (at $P < 0.05$) using SPSS Version-20.

Nine value-added products were developed by optimizing the amounts of ingredients and processing parameters such as boiling, steaming, refrigeration, drying, and soaking time. The RTC products were Tilapia & Tuna Fish balls, Pangas & Mackerel Fish sausage, Battered & breaded Tilapia & white Snapper fillet, Sardine Fish marinades, and RTE products were Tilapia & Tuna Fish crackers. The protein content of those 9 products were 11-19%, indicating protein-rich fishery products. In the case of shelf-life analysis, the MAP-3 (50% CO_2 & 50% O_2) gave the highest shelf life of 20 days and 16 days for Tilapia and Tuna Fish balls, respectively. On the other hand, MAP-1 (50% CO_2 & 50% N_2) gave the highest shelf life of 26 days for both Pangas and Mackerel Fish sausage. MAP-2 (40%CO_2, 30% N_2 & 30% O_2) demonstrated the highest shelf life of 16 days for both Battered & breaded Tilapia and white Snapper fillets. The highest shelf life of Sardine Fish marinades was 34 days under MAP-2 (40%CO_2, 30% N_2 & 30% O_2). MAP-1 (100% N_2) and MAP-2 (50% CO_2 & 50% N_2) both gave the highest shelf life of 5 and 6 months for Tilapia and Tuna Fish crackers, respectively. In the survey, a total of 43 consumers responded until now. Around 93% of respondents don't buy any value-added fish products. Most respondents (65%) didn't find any value-added products under chilled or refrigerated conditions. Almost all respondents preferred the pack design of purchased products. Finally, all respondents (100%) opined for introducing this kind of MAP packaged value-added fishery products in the market.

Processors now have access to the technologies for preparing RUFS, RTC, and RTE fishery-developed products, as well as information on their shelf life under various MAP packaging conditions. They can easily produce those convenient and quality products. However, further research needs to be conducted based on these technologies to foster small- and large-scale entrepreneurship.

Keywords: Ready-to-use fish products, Food security, Fish powder, Adolescent girls, RTC, RTE, Value-added, Shelf-life, MAP.

PBRG Sub-project Completion Report (PCR)

A. Sub-project description

1. Title of the PBRG sub-project

Development of Fish-based Food Products and Extension of Shelf-life to Enhance Nutritional Security.

2. Implementing organization (s):

Department of Fisheries and Marine Science, Noakhali Science and Technology University, Noakhali-3814 and Department of Fisheries, University of Rajshahi, Rajshahi-6295.

3. Name and full address with phone, cell and E-mail of Coordinator, Associate Coordinator, PI/Co-PI (s):

Name and address of the coordinators

15 Oct'19 to 09 Feb'22	*12 Apr'22 to 29 Jun'22*	*30 Jun'22 to 29 Dec'22*
Dr. Md. Monirul Islam Member Director Fisheries Division, BARC Farmgate, Dhaka, Phone: 01716-350628 E-mail: md-fisheries@barc.gov.bd	Dr. M. A. Awal Member Director Fisheries Division, BARC Farmgate, Dhaka, Phone: 01716-350628 E-mail: md-fisheries@barc.gov.bd	Dr. Md. Rafiqul Islam Director Nutrition Unit, BARC Farmgate, Dhaka, Phone: 01716-350628 E-mail: dir.nutrition@barc.gov.bd

Principal Investigators

Component-1 (NSTU)

Abdullah-Al Mamun, PhD
Professor, Dept. of Fisheries and Marine Science
Noakhali Science and Technology University
Cell: +8801712928710
E-mail: mamun_au22@yahoo.com

Component-2 (RU)

Dr. Md. Tariqul Islam
Professor, Department of Fisheries
University of Rajshahi, Rajshahi-6205
Phone: +88-01712697251,
Email: tariqrubd@gmail.com

Co-principal Investigators

Component-1

Md. Shahid Sarwar, PhD
Assoc. Professor, Department of Pharmacy
Noakhali Science and Technology University
Cell: +8801818280905
E-mail: pharma_sarwar@yahoo.com

Shuva Bhowmik
Asstt. Proffessor, Dept. of Fisheries and Marine Science
Noakhali Science and Technology University
Cell: +8801920591112
E-mail: shuva_bhowmik@yahoo.com

Component-2
Dr. Md. Yeamin Hossain
Professor, Department of Fisheries,
University of Rajshahi, Rajshahi-6205
Phone: +88-01751566077
Email: hossainyeamin@gmail.com

Dr. Syeda Nusrat Jahan
Assoc. Professor, Department of Fisheries
University of Rajshahi, Rajshahi-6205
Phone: +88-01737-000495
E-mail: nusratru@yahoo.com

4. Sub-project budget (Tk.)

4.1 Total original budget	:Tk.	27800000.00
A) BARC Component	:Tk.	6063000.00
B) NSTU component	:Tk.	8900000.00
C) RU component	:Tk.	12837000.00
4.2 Latest Revised (if any)	:Tk.	27537000.00
A) BARC Component	:Tk.	3063000.00
B) NSTU component	:Tk.	10065000.00
C) RU component	:Tk.	14409000.00

5 Duration of the sub-project

5.1. Start date (based on LoA signed) : 15 October 2019
5.2. End date : 29 December 2022

6. Background of the sub-project

Aquatic foods, 'seafood' is sought and enjoyed by people for cultural and gastronomic reason (Jennings et al., 2016) and can be a core part of peoples' diets. For instance, in Bangladesh fish provides 60% of animal protein (DoF, 2015). Fish and fishery products offer high value protein (USDA, 2012), essential micronutrients, vitamins, long-chain n-3 Polyunsaturated Fatty Acids (PUFA) (Karapanagiotidis, et al., 2006) and are, with some exceptions, low in saturated fats, carbohydrate and cholesterol (Sriket, Benjakul, Visessanguan, & Kijroongrojana, 2007). In particular, n-3 PUFA have valuable functions and have attracted considerable interest in their roles providing protection from Coronary Heart Disease (CHD) (Harris et al., 2009). Fish and seafood intake reduced mortality (Mozaffarian & Rimm, 2006). Eating 60 g fish/day reduced 12%

mortality (Zhao et al., 2016). Low diets of n-3 PUFA accounted death of 1.4 Million in 2010 and also responsible for roughly 1% of world's total burden of disease related disability-adjusted life years (DALY's) (Lim et al., 2012). Maintaining or increasing seafood consumption has been identified as a critical pathway to ensuring nutritional security. Although the estimated global fish consumption has gradually increased (OECD & FAO, 2015) and now dependent on sourcing from both fisheries and aquaculture, our understanding of the contextual factors affecting consumption by specific groups is often limited. Hidden hunger linked to micronutrient deficiency undermines progress towards the SDGs in a variety of ways. Sub-clinical deficiencies result in poor health and development of children; undermine reproductive health in adolescent and pregnant women resulting in intergenerational poverty. It may also undermine health, economic output and quality of life of older people. Thus, effective evidence-based interventions to improve the nutrition and nutrition-related health outcomes of vulnerable groups through enhanced dietary micronutrients rich Ready-to-eat product is important.

Bangladesh is a riverine country endowed with huge aquatic resources. Fish and fishery products are the major sources of animal origin diets of the people. Distribution, abundance, availability and affordability of fish varies with season, species, locality, and the purchasing capacity of consumers. Though aquaculture is growing rapidly, still the open water fisheries have enormous contribution to the total volume of fish production. During the dry season bulk amount of the fish are caught in the open water bodies (especially from *haor*, *baor*, seas, etc.) and are being underutilized for many reasons including poor storage capacity. This low-priced fish is destined to feed industry, dried fish industry and so on. However, fish price and availabilities are greatly varied over the year. Despite having huge aquatic resources, malnutrition still exists in Bangladesh where micronutrient deficiencies are the main concern. This so-called trash fish (mainly small indigenous fish species) is full of micronutrients and needed to supply adequately throughout the year to the vulnerable people. Apart from seasonality, heterogeneous food distribution at intra-household level deprived the vulnerable members such as female, adolescent and infant at household level. Ready-to-eat fish (prepared fish and fish products for consumption) based products like Fish powder could be a very good option to mitigate many challenges of food and nutritional security of vulnerable population. These products could be an alternative choice to utilize during lean period, as food aid for the population (for instance *Rohingya* immigrants from Myanmar, *Monga;* seasonal food crisis affected people in northern Bangladesh). Moreover, incorporating this powder into local cuisine will make it popular in the whole segment of the society.

Food preference has been changing with social and economic development of the country. Now, city-dwellers especially busy mothers and housewives seek Ready-to-cook (RTC) or prepared foods instead of raw ingredients in their busy life. Presently, there is no such value-added fishery products in the market. However, recently several companies in the country introduced some value-added meat products under frozen condition in the market. On the other hand, with increasing consumer demands for fresh products with extended shelf life and increasing energy costs associated with freezing and frozen storage, the fish-processing industry is actively seeking alternative methods of shelf life preservation and marketability of fresh, refrigerated fish and at the same time economizing on energy costs (Ashie *et al*. 1996).Therefore, value added products either Ready-to-cook (RTC) or Ready-to-eat (RTE) fishery products with sufficient shelf-life

under refrigerated condition could be introduced in the market. Fish is usually sold as whole or cut without proper storage and displaying facilities. As a result, a considerable number of raw fishes undergo quality deterioration resulting quantitative loss during retailing (Hossain *et al*. 2013). However, the retail superstores of the country now sell raw fishes or thawed fishes as whole or sometimes as steaks under refrigeration or icing condition. This chilled storage will not necessarily extend the shelf-life sufficiently for retail distribution and display purposes. However, repeated thawing and freezing is also practiced in superstores.

Modified atmosphere packaging (MAP) combined with low storage temperatures is an effective technique to achieve shelf-life extension of fishery products (Sivertsvik *et al*. 2002). MAP is a widely used packaging technique for displaying chilled fish, meat and their products in developed countries. It extends the shelf-life (25-400%) of the raw fish fillets at refrigeration temperature (Reddy *et al*. 1991; Pantaza *et al*. 2008). O_2 storage proved advantageous for maintaining the best hygienic conditions throughout processing and filleting as well as allowing for prolonged storage. Most researchers concluded that employing a higher level of CO_2 might extend the shelf-life of fresh fisheries products by 30 ± 60%. It allows retailers to sell raw fishes and their products at refrigerated condition for an extended period (Randell et al. 1995). In our previous study, the highest shelf-life of sliced tilapia was 15 days for MAP 1 (50%CO_2 & 50% N_2) followed by 12 days for MAP 2 (50% CO_2 & 50% O_2), 9-12 days for vacuum pack and 6-9 days for no pack (control) sample (Karim et al. 2020). In case Rohu fish, the highest shelf-life was 16 days for MAP 1 (50%CO_2 & 50%N_2) followed by 13 days for MAP 2 (50% CO_2 & 50% O_2), 11 days for vacuum pack and 8 days for no pack (control) sample (Das et al. 2021). On the other hand, the highest shelf-life of sliced Goonch fish (Baghair) was 12 days for MAP 1 (50%CO_2 & 50%N_2) followed by 10 days for vacuum pack, 9 days for MAP 2 (50% CO_2 & 50% O_2), and 6 days for no pack (control) sample (Alice et al. 2020). However, vacuum packaging significantly increased the shelf-life, but it is not suitable for all types of fishery products particularly for soft and breakable products, as the pressure created during vacuum packaging can break down the products structure inside the pack. Therefore, MAP packaging with thermoformed tray (using Tray Sealer) instead of pouch is more convenient for displaying the products for the consumer. Previously, several scientists attempted to develop different types of value-added fishery products in the country including Fish ball, Fish finger, Fish mince, Fish burger, Fish cutlet etc, which can be stored at freezing and refrigeration temperature (Hoque et al. 2007, Nowsad et al. 2007, Flowra, 2013, Zzaman *et al*. 2017). However, no study was found to increase the shelf-life of those value-added products by the combination of MAP packaging and refrigerated storage condition. Therefore, there is a scope to produce different value-added products from low value fish and preserve those fishery products under MAP packaging condition in different storage condition to attain maximum shelf-life for marketing in the superstores. In Bangladesh, this type of packaging system has not developed yet in the market. Therefore, development of different value-added products either RTC or RTE and preservation through MAP packaging and refrigerated storage is required to ensure the supply of quality fishery products with the extended shelf life in the market. Additionally, consumer's acceptability of the prepared products needs to be assessed in field situation particularly in retail superstores.

7. Sub-project general objective (s)

Development of fish-based value added and Ready to use supplementary food products (RUSFs) to ensure food and nutritional security for different segments of people.

- Mapping seafood consumption pattern and nutritional status of adolescent girls at different geographical areas of Bangladesh

- Identification of the most suitable fish species and formulate Ready-to-use fish products to minimize the nutritional requirement gaps.
- Development of marketable value-added fishery products and shelf-life extension through MAP packaging to increase the value, convenience and acceptability of fishery products in the market.

8. Sub-project specific objectives (component wise)

A) Coordination component (BARC)

i) to ensure smooth and efficient implementation of sub-project activities to achieve desired project outputs within the stipulated timeframe under strengthened capable research management system;
ii) to coordinate project implementation efforts and integration of activities to generate desired information /technology as per methodology of the sub-projects;
iii) Identify operational deviations and addressing constraints/problems (if any) under a process of strong and regular monitoring of the project activities;
iv) to upgrading the level of output of the sub-project through reviewing of yearly technical progress;
v) Collect and collate project data, finding and observation and production of compiled Project Completion (PCR);

B) Component-1 (NSTU)

i. To know the intra-household food consumption, food allocation and nutritional outcomes of adolescent girls through baseline survey;
ii. Development of Ready to use fish-based nutrition-dense products using most suited fish species on the basis of nutritional quality, affordability and availability and assess their quality, appearance and microbial load;
iii. Screening the best suited fish-based products on the basis of different nutritional, microbial, toxicology and sensory evaluation and fit in the best products into the local cuisine with an appropriate ratio of fish and vegetable/legumes to minimize the nutritional gaps obtained from the baseline survey;

C) Component-1 (RU)

i. To develop the Ready-to-cook (RTC) (Fish ball, Battered & breaded fish, Fish marinades, Fish sausage) and Ready-to-eat (RTE) (Fish crackers) from freshwater and marine fish;
ii. To determine the overall shelf-life of RTC and RTE products stored under modified atmosphere packaging at different storage temperature;
iii. To know the consumer's acceptability of MAP packaged fishery products in the superstores of the Dhaka city;

9. Implementing location (s)

A. Component-1 (NSTU): Noakhali, Lakshmipur and Khagrachari districts;

B. Component-2 (RU) : Department of Fisheries, University of Rajshahi; selected superstores of Dhaka and Rajshahi city

10. Methodology

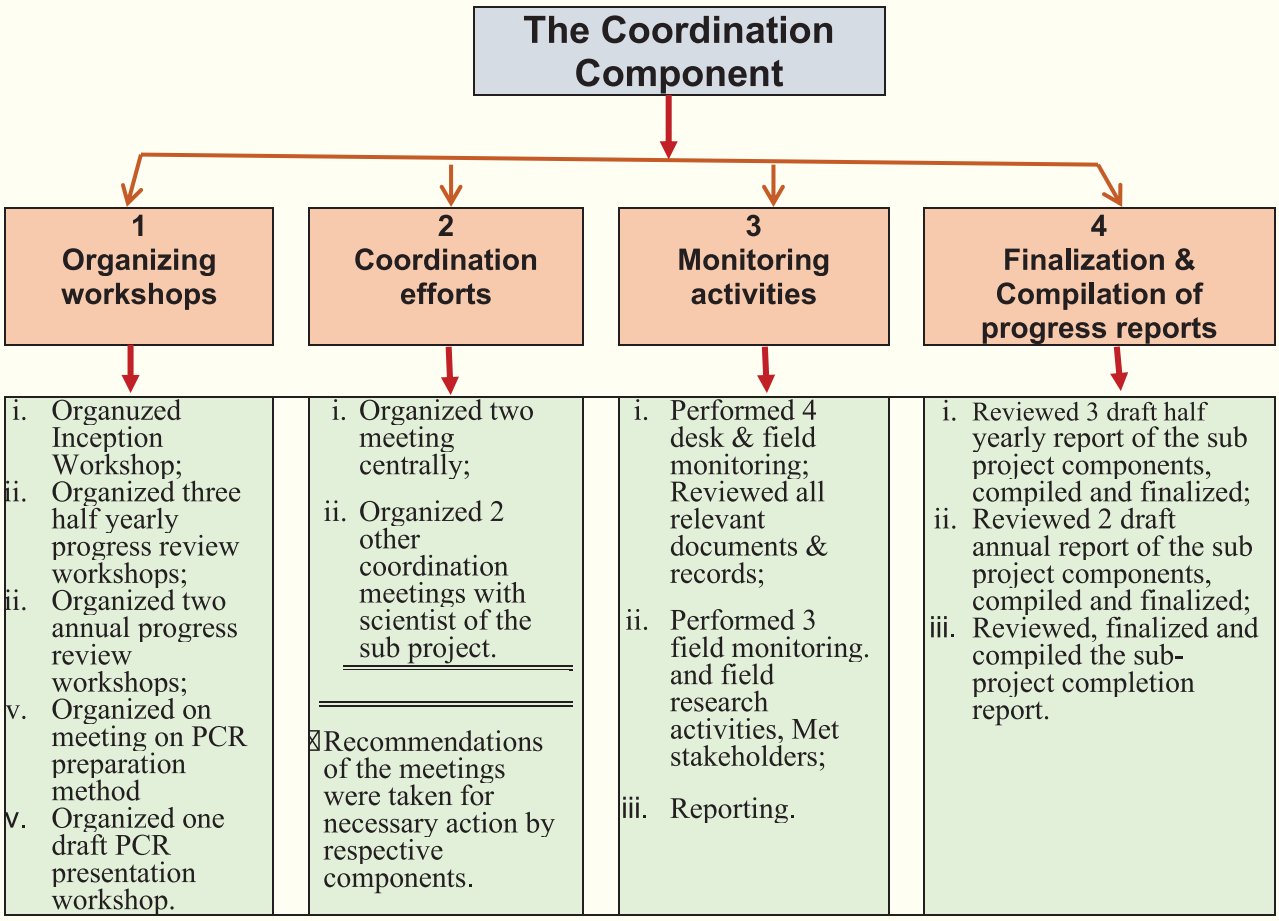
10.1 Activity implementation approach of the Coordination component (BARC)

The Coordination component as the responsible unit of the sub project to initiate all potential efforts in the process of implementation of each component under the sub project so that the general objectives and goal of the sub project can be achieved through smooth and successful completion of each of the specific objectives as per activity time plan of the sub-project document. To ensure that, the Coordination component, taking into consideration its activity and objectives and duration of the sub-project, thus accordingly designed its plan of activity (approach) for the proposed period.

Following are the major activities carried out by the coordination componen:

a. Organizing seminars/workshops.
b. Monitoring of the sub-project activities (specifically financial and research activities);
c. Coordination of activities within the components of the sub-projects.
d. Review and compilation of half yearly and annual research progress reports.

The implementation approach and activities there under the Coordination component of the sub-project are shown in the following diagram:



Recommendations of the inception workshop, half yearly and annual research progress review workshops and different coordination meetings are furnished hereunder in **Annexures: BARC (A – E).**

Following table presenting the summary statement of achievements performed by the Coordination component of the sub project:

Summary statement of achievements		
Name of activities	**Performance against each component of the sub-project**	**Remark**
Inception workshop	Organized centrally at BARC in November' 2018	Attended all PI, Co-PI & expert members.
Revision of PP	Done as per recommendations of inception workshop	
Half yearly progress review Workshop	Organized centrally at BARC in March 2020, January 2021 & June 2022.	Attended all PI, Co-PI & expert members
Workshop on Draft PCR	01 (24-25 Oct'22)	Review of the respective draft PCR done
Ann. Progress review Workshop	Organized centrally at BARC in December 2020 & in April 2022	Attended all PI, Co-PI & expert members.
Coordination meetings - 03	04 *(Two meetings held virtually)*	All coordination meeting held centrally.
Monitoring of field and Lab activities	04 *(NSTU & RU)*	Covered all components under the sub-project.
Training/orientation	01 (11.05.22)	Orientation workshop of 09 sub-project PIs on PCR development
Financial achievement	Approx. 91% of total approved budget and 100% of released money	Delay in procurement plan approval and Covid-19 pandemic hampered desired progress
Reporting performance	Provided sub-project inception report, SoE, Half yearly and Annual compiled progress reports of all sub project components as per planned time frame.	Major reports/proceedings: • Inception report (1 no); • Compiled half yearly progress report (3 no); • Compiled annual progress report (2 no); • Monitoring reports (4 no); • Workshop proceeding (6); • Project Completion Report (1);

Plate 1. Pictorial views of different workshops, coordination meetings and field monitoring activities

10.2 Component-1 (NSTU)

Component-1 was designed with multiple objectives. They were pursued in chronological manner; i.e., Fish powder (FP) development and receipe development, baseline survey and endline survey on households with FP Intervention, consumer preferences of FP, Mice Model analysis for FP nutritional evaluation, shelf-life prediction of FP storage. For having a clear picture of all research activities related to component-1(NSTU), the methodologies are presented in Fig 1.

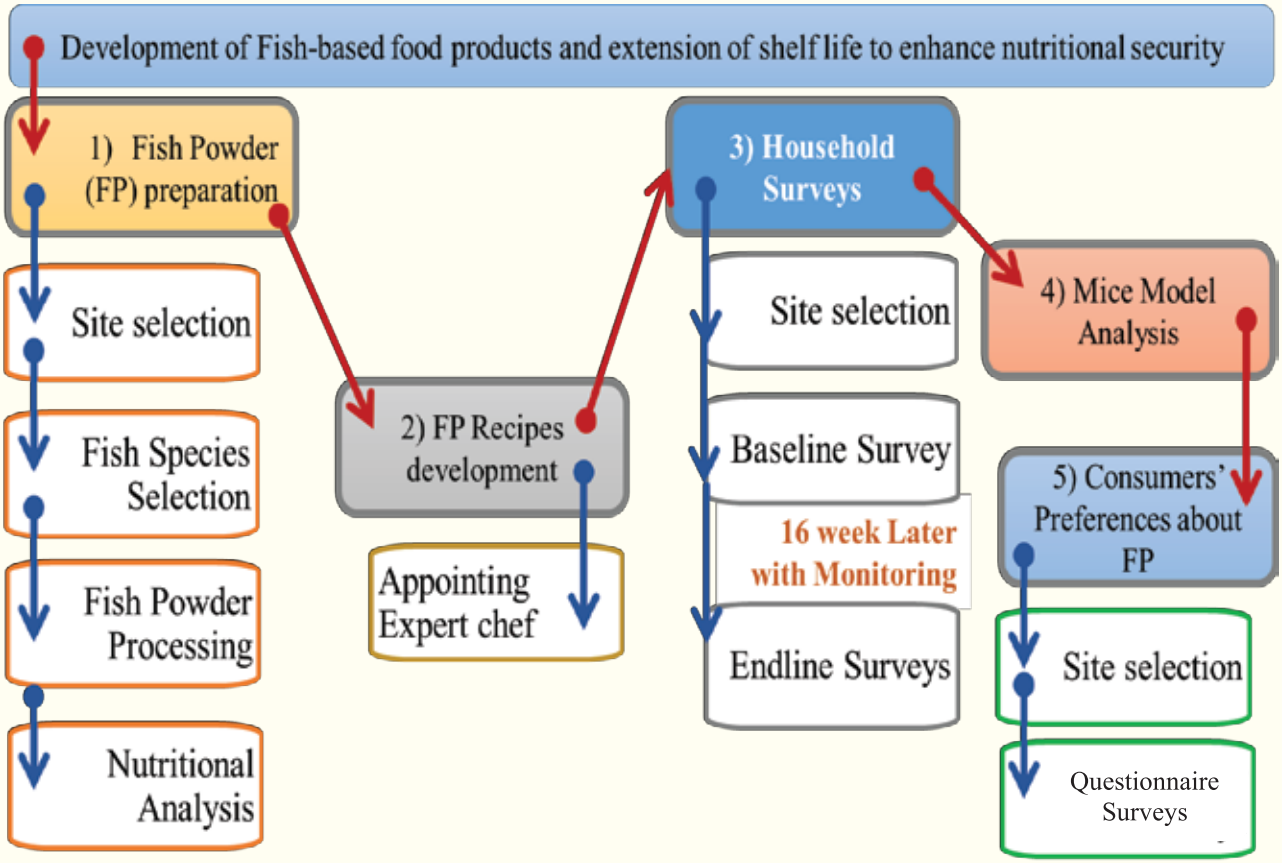


Fig 1. Flow diagram of the whole methodology

10.2.1 Fish powder preparation and development of Fish powder-based recipes

10.2.1.1 Fish species selection

Fish species were selected on the basis of food nutrition and security (FNS) criteria. Every fish species was selected according to their market availability, consumers' affordability, its nutritional status and desirability or acceptability (Fig 2). Five low-cost, nutrient-dense fish were selected and collected for the preparation of RUFPs. Indian River shad (*Gudusia chapra*, Chapila) and Ganges river sprat (*Corica soborna*, Kachki) were collected from the Kaptai Lake, Rangamati, whereas Ticto barb (*Puntius ticto*, Tit punti) was collected from the haor areas of Kishoreganj (Fig 3). Pointed tail Anchovy (*Coilia dussumieri*) and fringe scale Sardine (*Sardinella fimbriata*) deceased fish samples were purchased from the fish landing centre of Cox's Bazar, Bangladesh (Fig 3).

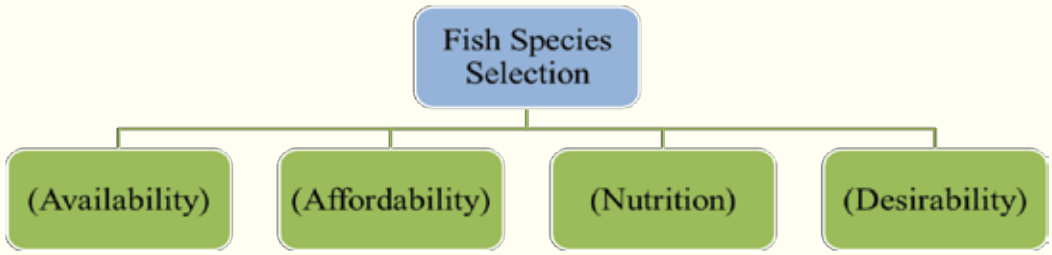


Fig 2. Criterion for the fish species collection for Fish powder preparation

i. Sampling procedure

Small-sized fish were mainly obtained from the local small-scale fishers. Both Chapila and Kachki from Kaptai Lake, Sardine and Olua from fish harbor of Cox's bazar and Ticto Barb from Kishoreganj Haor were collected. Immediately after harvesting fish were kept in the icing and to maintain cooling temperature continuously adequate ice were added and it was 1:10 (fish: ice). Insulated iceboxes were used to ship the samples from the site to the lab. Fish were sorted and kept in ice box immediately after reaching to the harbor/landing centers. It took seven hours at night to reach NSTU from Cox's bazar. While reaching at NSTU the temperature of the box was checked and it was found more than 50% of the ice remained intact. Fish kept in the lab freezer accordingly.

Individual fish in the pooled sample were verified as species and weight. Local name, English name, scientific name, and location of sample collection were noted accordingly. The individual length and weight of the fishes were recorded as well.

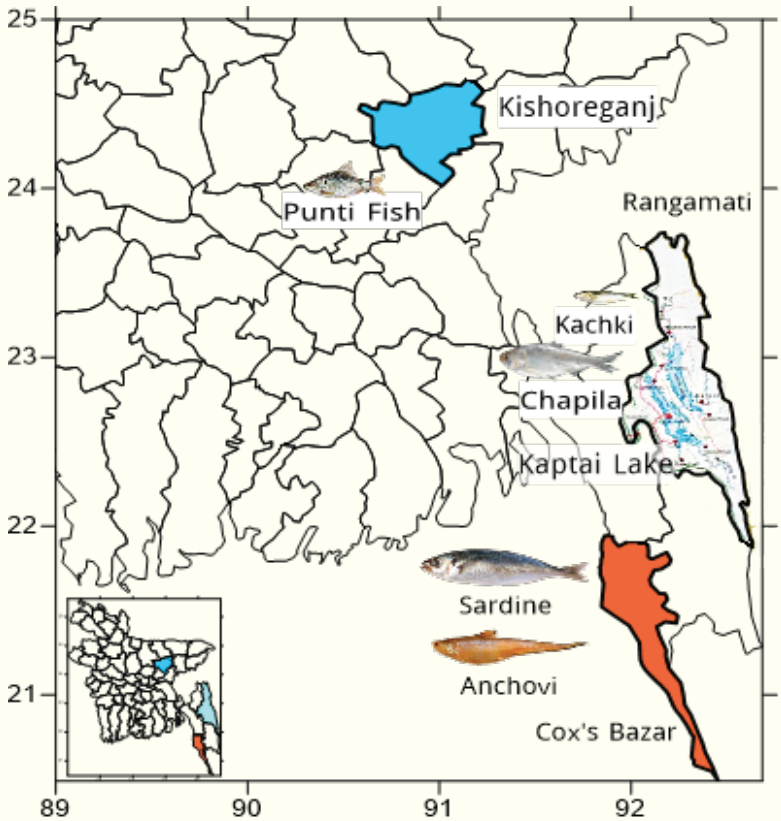


Fig 3. Fish species collection sites for Ready to use fish products (Fish powder) development

A unique code number was used for each specimen within a pooled sample. The code numbers were written on the top of each transparent polyethene bag and then recorded in a notebook. These samples were carried in a portable cooler and then transferred in the refrigerator at -18°C in the NSTU lab. During the whole process of sample collection and transfer from one place to another, a standard chain of custody was maintained according to Bogard et al., (2015) and Mamun (2017). During the shipment in the proposed lab, adequate amounts of dried ice were used and the temperatures were checked in both ends of sending and receiving points.

ii. Sample preparation

Precautionary measures were taken to avoid all possible cross contamination of non-metal materials including plastics. Fish samples were washed properly with deionized water and then transferred for packing with polythene bag. During this washing process, fish samples were maintained in the cool chain (fish samples were kept in an ice box with adequate ice at least ice: fish is 10:1). After processing and drying fish 50-100 g in each poly bag were kept with appropriate code number on the top of the bag for nutrient analysis. After packing, fish samples were stored in deep freezer at -18°C. Then the samples were shipped to the respective laboratories of South Korea, UK and local lab for further analysis following Karapanagiotidis et al (2010).

Ready-to-use dry fish for Fish powder production in safe and hygienic procedure

Ready-to-eat safe and hygienic preparation of fish powder and seaweeds products

Ready-to-use food products (RUFPs) incorporated in local recipes

Plate 2. Schematic concept of current research; from dry fish to delicate recipies followed by Fish powder

10.2.1.2 Preparation of edible Fish powder

Fish samples were divided into three sub-groups for the development of whole, gutted, and muscle RUFPs. To prepare Fish powder from the whole fish, samples were completely washed with water to eliminate dirt, dust, and other foreign matter (Plate 2). Descaling and eviscerating fish were followed by using a clean work table in hygienic conditions with the help of sterilised tools for the preparation of gutted Fish powder. The fish fillet was separated by using a sterile blade for preparing muscle powder. Then, whole fish, gutted fish, and muscle were subjected to sun and oven drying. For sun drying, the whole fish, gutted fish and muscle were kept at 25–30°C for 5, 3, and 2 days (8 h per day) respectively. Thereafter, they were kept at 65°C in electrical oven (VZLG-9620, EJER TECH, China) for 2 days, 1 day, and 18 h, respectively. After drying, the

whole fish, gutted fish, and muscle were ground by using an electric grinder (Chef Pro-MG 128, Preethi, India) into a fine powder (500-micron mesh). The ground powder was packaged with high-density polyethylene and stored at 4ºC for nutritional analysis.

10.2.1.3 Nutrient's content analysis of prepared Fish powder

i. Proximate composition analysis

The gross chemical compositions of the fish and by-products were determined by the proximate analysis based on official methods of the Association of Official Analytical Chemists (AOAC, 2000). All samples were determined directly from the wet samples where selenium and energy content were determined from the dry sample (Table 1). The samples were finally ground and blended homogeneously before analysis.

ii. Moisture content (drying oven)

The moisture content of samples was determined by placing 1 g of a wet sample in a drying oven at 110 °C overnight (AOAC, 2000).

iii. Crude protein content

The crude protein content was determined from the nitrogen content of each aquatic animal samples which assumes that protein contains 16% nitrogen, using automated Kjeldahl analysis (Tecator Kjeltec TM 2300 analyser, Foss, Warrington, UK) according to the standard method (AOAC, 2000) and the manufacture's protocol.

iv. Crude lipid content

Total lipid for fatty acids analyses was extracted from the fish flesh and by-products by homogenisation in chloroform-methanol according to a standard protocol (Folch et al., 1957). The total lipid was re-dissolved in chloroform: methanol (2:1 v/v) + containing 0.01% (w/v) BHT to a concentration of 10 mg/ml and stored in nitrogen at -20 °C for further analysis.

v. Ash content

The inorganic matter or total ash content of samples was determined by placing a 1 g wet sample into a muffle furnace at 600°C for 16 h (AOAC, 2000).

vi. Energy content

The gross energy content of fish and by-products samples were determined by bomb calorimeter (Parr© 6200; Foss, Warrington, UK) where the dried samples were completely combusted in an oxygen filled container and the heat released measured and energy content calculated according to manufacturer's protocol (Pratoomyot, 2010).

Table 1. The analytical methods and references followed for each nutrient component analysis of aquatic animals in S-W Bangladesh

Parameter	Unit	Methods used	References
Protein	g/100 g	Automated Kjeldahl analysis (Tecator Kjeltec TM 2300 analyser, Foss, Warrington, UK)	(Karapanagiotidis et al., 2006)
Total lipid	g/100 g	Folch	(Folch et al., 1957)
Moisture	g/100 g	Air drying (AOAC 1990-942.05)	(Karapanagiotidis et al., 2006)
Ash	g/100 g	AOAC 1990-942.05	(Karapanagiotidis et al., 2006)
Energy	KJ/g	Bomb calorimeter (Foss, Warrington, UK)	(Doyle et al., 2007)
Fatty acids	g/100 g	GLC	(Karapanagiotidis et al., 2006)
Micronutrients	µg/100 g	Acid digestion, ICP MS	(Wheal et al., 2016) (Erkan & Özden, 2007)
Selenium	µg/100 g	ICP MS	(Silva et al., 2011)

vii. Micronutrients

The micronutrients were determined in acid digestion ICP-MS (Inductive Coupled Plasma-Mass Spectrometry) (Erkan & Özden, 2007; Wheal et al., 2016). Regarding selenium determination, ICP-MS without acid digestion was used (Silva et al., 2011). Wet samples were used for all of the micronutrients tests, however, for selenium dried (ash) samples were used and then data converted to wet weight basis (Poppe et. a. 2007). The minerals (Na, Mg, P, K, Ca, Cr, Mn, Fe, Co, Cu, Zn, Se, As, Cd, Pb, V and Ni) and fatty acid composition of developed powders were evaluated at the Nutritional Analytical Laboratory, Institute of Aquaculture, and University of Stirling, UK. The minerals value determined by the using of inductively coupled plasma mass spectrometry (ICP-MS) with collision cell technology (Thermo X, Series 2, Thermo Scientific, UK) according to Sprague et al. 2020. A 25 mg powder samples were digested with 5 mL 69% nitric acid (Aristar® analytical grade, VWR chemicals, UK) in a microwave digester (MARS Xpress, CEM Microwave Technology Ltd., UK) prior to analysis by ICP-MS. The operating mode of ICP-MS was kinetic energy discrimination (KED) and 100% Helium gas used as collision to correct for interference. Argon is used as the plasma gas, while scandium and gallium are considered internal standards.

viii. Fatty acid analysis

Fatty Acid Methyl Esters (FAME) were obtained from total lipid subjected to acid catalysed transesterification according to a standard method and quantified by gas chromatography (Christie, 2003). The procedure for fatty acids analysis was carried out according to the protocols developed/followed by the Nutritional Analytical Service (NAS), Institute of Aquaculture, University of Stirling, UK (Pratoomyot, 2010).

Gas liquid chromatography (GLC) was used to determine the composition of fatty acid. Fatty acid methyl esters (FAME) were obtained from lipid by acid catalyzed trans esterification at 50ºC for 16h by using 2 mL of a 1% (v/v) solution of sulphuric acid (95% Aristar® analytical grade, VWR chemicals, UK) in methanol and 1 mL toluene. FAME were separated by GLC using a Fisons GC-8160 (Thermo Scientific, Italy) equipped with a ZB-wax column (30 m x 0.32 mm i.d. x 0.25 um). The hydrogen used as the carrier gas and the operating conditions were the same as described by Sprague et al. 2020.

ix. Amino acid analysis

The amino acid profile of developed Ready-to-use Fish powders were assessed at the Regional Analytical Science, Korea Basic Science Institute (KBSI), Seoul Center, South Korea by the using of validated an Agilent 1260 series (Agilent Technologies, Walbronn, Germany) high performance liquid chromatography (HPLC), followed by Desai et al. 2018. A total of 26 mg powder samples was digested in 6 N 1 mL of HCl (95% Aristar® analytical grade, VWR chemicals, UK) in an oven at 110ºC for 20 h. The operating conditions were Waters Nova-Pak C18 um column (3.9 x 300 mm), 46ºC column oven temperature, Agilent 1260 series binary HPLC pump and auto-sampler injector, HP 1100 series, 254 nm variable wavelength detector, 1 mL/min flow rate, 30 min run time, 10 min equilibrium time and 10 uL was injection volume. The results are articulated as mg/g protein of powder sample.

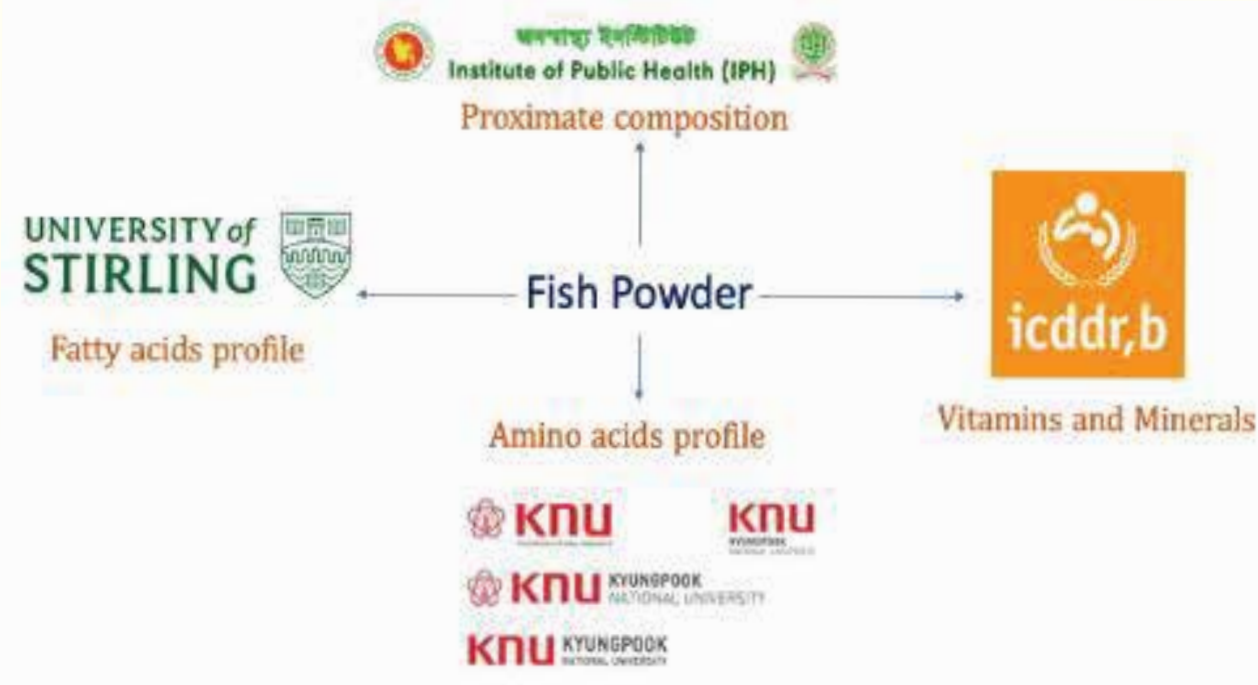


Fig 4. Institutes involved in Fish powder nutrition and biomarker study

10.2.1.4. Calculation of potential contribution to RNI

The recommended nutrient intake (RNI) was calculated using the reference value of each nutrient for adults (A), infants (I), and pregnant and lactating women (PLW). The standard portions of Chapala, Punti, and Kachki powders are 15 g/day, 12 g/day, and 8 g/day for adults, infants, and pregnant and lactating women, respectively. Nutritional parameters (Ca, Se, Zn, Fe, protein, fat, and LC n-3 PUFA) were selected for the percentage of RNI analysis.

10.2.1.5. Statistical analysis

All the evaluated data were presented as mean ± standard deviation (SD). Statistical analysis was done by using statistical package for the social sciences (SPSS) version 20 (SPSS Inc., Chicago, USA). DMRT (Duncun Multiple Range Test) was used to know the significant differences among samples ($p < 0.05$).

10.2.1.6 Food recipe preparation with Fish powder

Before jumping into large scale Fish powder preparation, we have collected fish from the local markets and then sun dried at NSTU campus. We brought a small blending machine.

Fig 5. Fish powder grinder

We prepared powder of local fish species collected from the local markets. These powders were then sent to the celebrity chefs and other female entrepreneurs in the town. They tried and made some recipes.

Plate 3. Raw and dried fish ready for powder preparation

Initially, small amount of fish (20-25 kg in wet weight basis) were collected from the local fish market and sun dried in NSTU campus. The dried fish then made powder by using a simple grinding protocol (Fig 6)

Fig 6. Pictorial presentation of different steps of powder preparation with locally available species at experimental stage

For the later part of the experiment, we bought targeted five fish species and made three-sub groups (whole, gutted and muscle) of each species except Kachki fish. These 13 fish types were then dried at different ways to prepare Ready-to-eat (RTE) cooked products. We got more or less similar pattern of taste, smell, and colour from each type of prepared Fish powder. Then on the basis of nutrients of Chapala (retrieved from literatures) was selected for large scale trail.

Table 2. Some key nutrients in 100 g dry Fish Powder of Chapila fish (Source: Bogard et al. 2015)

Biomarkers	Vit-A	Vit-B12	Fe	Ca
Unit	µg	µg	mg/100 g	mg/100g
Amount	292	28	30.4	4252

i. Selection of Fish powder type

It was expected that the selected Fish powder would be economically viable as it only requires a thorough washing and no other losses arises as byproducts. The nutrient contents were also expected to be higher in intestinal tract, intramuscular bones, eyes and head portion along with key essential micronutrients. Among the three types of Chapila powder (whole, gutted and muscle) the best one was selected through a panel test process. The selected chefs were asked to cook same food item to find the best fitted Chapila powder. After cooking they ran the panel tests independently and didn't notice any significant differences among these three types of Chapila Fish powder. In the meantime, Chapila powder was also distributed among the female faculty of NSTU as well as female students to prepare recipes at home. Female faculty members and students also made different types of recipes and suggested the team in various ways. Some suggested for pre-cooking (fry) powder and then to add in the main recipes. Some hotel and restaurants also receive powder and they also tried the same. One of the key restaurants in the town "Nice guest house™" made a special cutlet with Chapila Fish powder.

ii. Recipe standardization

For cutlet and other mesh (locally called vorta), whole Fish powder were used as per the portion size along with onion, chilli, oil and other required ingredients. However, in order to prepare recipes, it is essential to standardize the quantity of Fish powder to be added to curry. Chefs used 30g, 40g and 50 g Chapila Fish powder to cook a curry for five-person meal. After a series of panel test it was observed that 40 g Chapila Fish powder is suitable in all aspects of smell, colour and taste. Selected chefs, students and female faculty members have prepared dozens of recipes and after a series of test seven recipes have been selected to standardize and circulated among the end users.

Plate 4. Trail phases of curry preparation with Fish powder

10.2.1.7 Preparation of extension materials

The standardized seven recipes were planned to record with celebrity chefs however the movement restrictions due to COVID-19 pandemic halted and had to organize locally. A local celebrity chef also the owner of "Box in the town" Afrin Chowdhury (Plate 5) were invited to showcase the products. All the recipes preparation modules were recorded on video and also formula enlisted in the leaflet in local language. Both videos and leaflet (in Bangla) were then used as extension materials to acquaintance people with the Fish powder-based recipes. Seven video clips for the seven recipes were prepared and uploaded in a dedicated YouTube™ channel. At least 1000 pieces of colored leaflet were prepared in local language.

Plate 5. Expert Chef appointed for developing delicate recipes

Mash preparation with Chapila fish Powder

Bringal Fry with Chapila fish Powder

Beet stalks preparation with Chapila fish Powder

Kalmi Spinach preparation with Chapila fish Powder

Mere trifle preparation with Chapila fish Powder

Cutlet preparation with Chapila fish Powder

Plate 6. Preparation of recipes using Fish powder by expert chefs

10.2.1.8 Mass Fish powder production

Chapila fish were collected from the vendor based in Rangamati and fish were collected every 15 days interval to get fresh and quality dried fish.

Plate 7. Fish drying with care on hanging shelf in Kaptai Lake

After proper packaging dried fish were transported from Rangamati to NSTU. After collection fish quality were checked and then dried both in oven and sun. After a final sorting dried fish were grinded in a large-scale machine. The machine can produce at least 20 kg Fish powder in an hour.

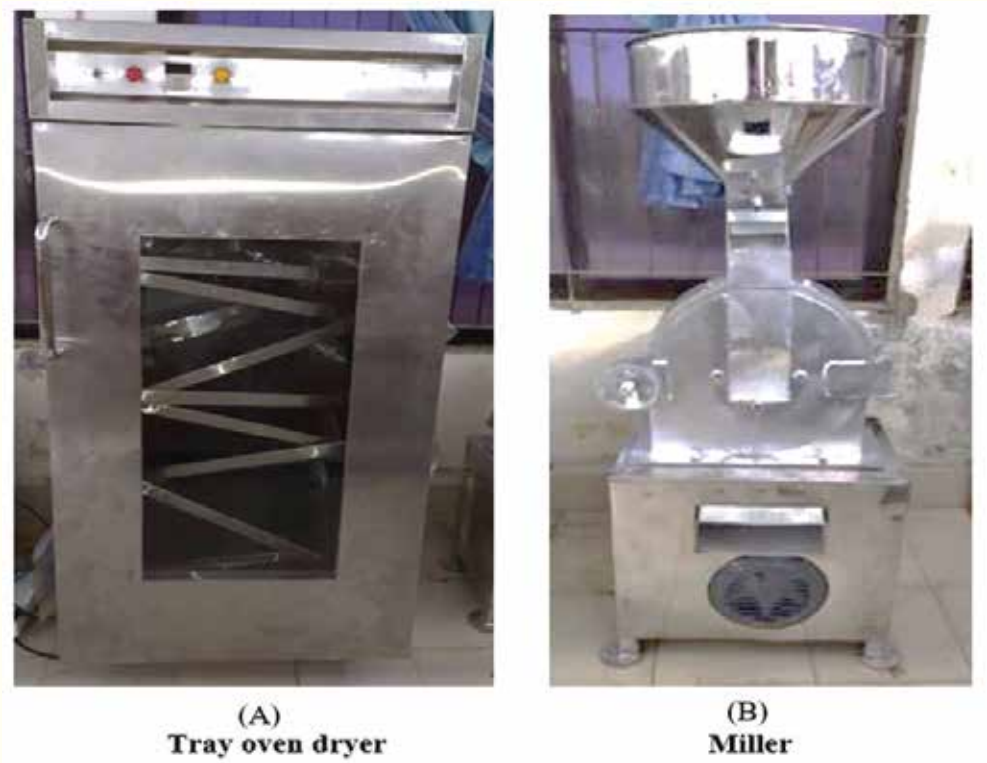


Fig 7. Tray oven dryer for wet fish drying (a) and dried fish miller (b)used for Fish powder production

10.2.1.9 Packaging and preservation

Immediately after grinding Fish powder were sieved properly and then packaged in small plastic pouch. Each plastic pouch contained 200-250 g Fish powder. It was designed such a way to provide 40 g Fish powder to each household, member per week during intervention. For instance, a family comprised five members got a 200g pouch for a week during intervention. For each intervention site team used to supply Fish powder for one week in a medium size box (200-250g*40 number).

Fig 8. Fish powder packaging for distribution.

10.2.1.10 Training session for the enumerators

To execute the socio-economic study, both male (n=3), female (n=10) enumerators were selected from Noakhali Science and Technology University. One male and one female health technologist were recruited from the local hospital of Maijdee, Noakhali. All the enumerators were postgraduate students from fisheries and Pharmacy backgrounds. Health technologists were experienced in blood collection were competent for the assigned job. Both classroom-based and in field training (Pretty, 1995) were provided to the enumerators. Experts from the relevant disciplines were invited to provide hands-on training. Researchers and demonstrators were actively present at the field level to monitor and support the field enumerators. An initial pilot survey and then model data were collected from the adjacent village (Bangla Bazar) NSTU and presented in the interactive reflection session (Plate 8).

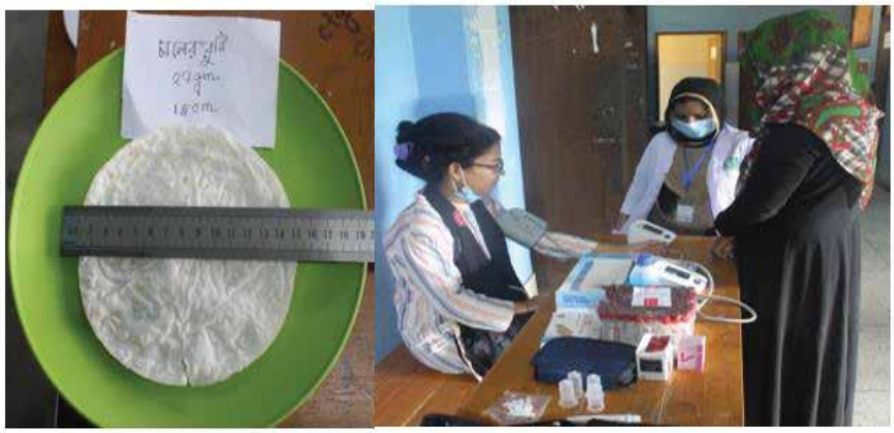


Plate 8. Comprehensive training among enumerators and health technologist.

A number of comprehensive trainings were participated by the staff and project workers for better understanding the procedures and questionnaire. Several actions have been performed to service the issue before the community despite disregarding all scientific evidence in its original form.

Plate 9. Training and workshop in the presence of honorable Vice-Chancellor and Treasurer of NSTU

Emphasis was also given to appropriate attitudes, behaviour (Pretty, 1995) local customs and researchers self-awareness (Morales, 2007). Support and the knowledge about the local context and customs were obtained from local NGO's staff.

10.2.1.11 Site selection

The study was conducted at three different regions (Noakhali, Lakshmipur, and Khagrachari) of Bangladesh (Fig 9). In this investigation, a total of 240 samples were gathered from three sites, each of which contained 80 adolescent girls (40 intervention and 40 control). A number of meetings with various stakeholders in each location were held before to the starting the field investigation.

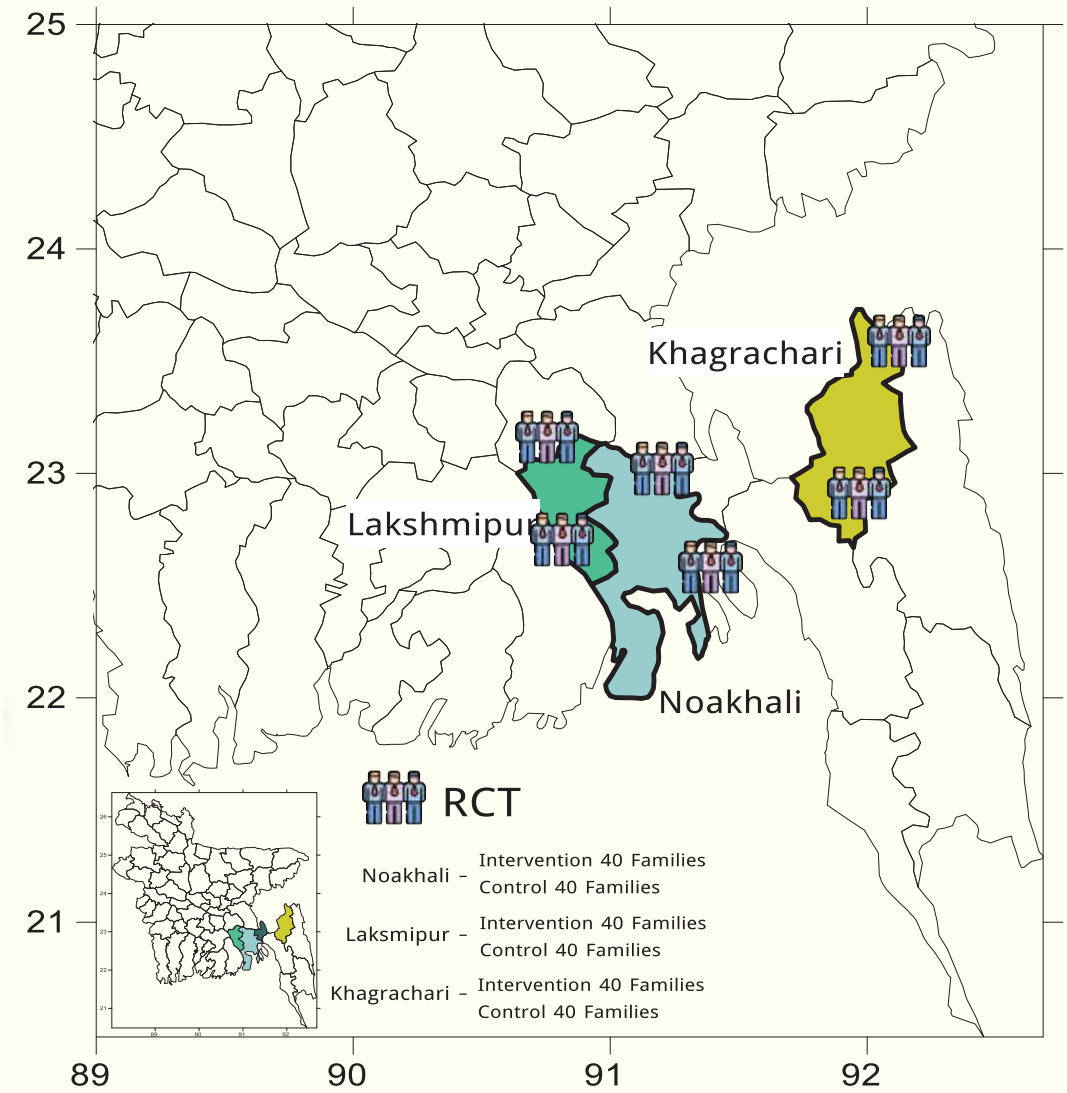


Fig 9. Six communities in three regions (**A:** Noakhali; **B:** Lakshmipur; **C:** Khagrachari) selected for the RCT of Fish Powder

10.2.1.12 Participatory mapping

In research and development activities participatory mapping is another popular, widely used PRA tool that can be used in diverse ways (Chambers, 2008). The community people enjoyed the mapping practice, and it allows for building rapport with the researcher which ultimately helped

to explore in-depth information to a greater extent. The use of participatory mapping was found to be satisfactory in Bangladesh for aquaculture research (Faruk-ul-islam, 2007). In the current piece of work, the development workers who worked with the community in the long term helped to find out the key informants, and geographical boundaries of the community. The community people in this study enjoyed the mapping practice and developed the village resource map.

Fig 10. Drawing maps of resources by local participants.

10.2.1.13 Well-being practice

Household level well-being ranking is widely accepted and replaced the traditional wealth ranking interview survey in many developments and research program (Scoones, 1998). Well-being indicators included social, education, health, culture and wealth (Stirrat, 2003). This technique is suitable for rapid identification of the lowest income group, stratification of social levels and households status in a given community (Chambers, 1994). It is also a useful tool where community people can exercise the social stratification in a realistic way (Pretty, 1995). In the recent aquaculture impact studies in Bangladesh, well-being ranking was widely used (Haque, 2007; Karim, 2006).

Plate 10. Site selection in Noakhali, Lakshmipur, and Khagrachari

In this study well-being exercise brings the livelihood context of the community people using well-being status based on key informant's (KI's) interviews and focus group discussions (FGDs). The primary objective was to understand the stratified community people by their capital and household resources. A collaborative approach was undertaken among the researcher, facilitators, enumerators and key informants. The KI's selection was executed with the help of local facilitators. At least three key informants from each site were selected similarly to Morales (2007).

Plate 11. Social well-being data collection and ranking practice at community level

Local leaders, matbbor (head of the community), school teacher and most knowledgeable person were selected as the KI's. The KI's of course remained in the area for most of the time (Mukherjee, 1997).

In the first meeting with the KI's the scope and goal of the research work was described clearly by the researcher and sought the neutral and unanimous information for quality work. A list of the households was developed first with the active participation of the key informants and most recent voter list (comprising of people over 18 years old living in the particular locality). This helped to ensure the inclusion of all the members of the community. The well-being ranking was then executed according to other researchers in aquaculture (Haque, 2007; Morales, 2007). The KI's got the freedom to group individual households (Keough, 2014). Finally, each of the households obtained three scores from the respective KI's and then average scores were calculated and grouped into four different categories with natural breaks of score viz. rich, medium, poor and ultra-poor. This type of well-being studies was practised in Bangladesh and proved useful in field research (Haque, 2007; Lewis, 1997).

i. Household selection for in-depth survey

Households from each social well-being group were selected purposely, and the criteria were selected households having at least one unmarried adolescent girl (10-19 years old). On this ground, at least 40 households from each community (40 HH×2 arms [intervention, control] × 3 [areas; Noakhali, Lakshmipur and Khagrachari=240 HH) were selected. Intra-household food allocations, nutritional outcomes of the adolescent girls are derived from the in-depth survey.

ii. Sample size calculation

We used the equation needed to compare 2 means: 2-sample, 2-sided equality for the calculation of sample size. This equation is useful for tests concerning whether the means of two groups are different. Two groups are 'Intervention (A)' and 'Control (B)' group and we collect samples from both groups i.e., we have two samples. We perform a two-sample test to determine whether the mean in group A, μ_A, is different from the mean in group B, μ_B. The hypotheses are:

$$H_0: \mu_A - \mu_B = 0$$

$$H_1: \mu A - \mu B \neq 0$$

where the ratio between the sample sizes of the two groups is

$$\kappa = nA/nB$$

This calculation uses the following formulas to compute sample size and power, respectively:

$$n_A = \kappa n_B \text{ and } n_B = (1+1/\kappa)\,[\sigma(z_{1-\alpha/2}+z_{1-\beta})/(\mu_A-\mu_B)]^2$$

$$1-\beta = \Phi\,(z-z_{1-\alpha/2}) + \Phi\,(-z-z_{1-\alpha/2}),\ z = (\mu_A-\mu_B)/\sigma\sqrt{}\,(1/n_A+1/n_B)$$

Where,

- $\kappa = n_A/n_B$ is the matching ratio
- σ is standard deviation
- Φ is the standard Normal distribution function
- $\Phi-1$ is the standard Normal quantile function
- α is Type I error
- β is Type II error, meaning $1-\beta$ is power

Table 3. Sample size estimation for different biomarkers

Parameter	Placebo (Literature)	SD	Treatment	Sample size
Hb	12 g/dL	4.5	13.8 g/dL	226
Vit A	1.04 µmol/L	0.34	1.2 µmol/L	163
Ferritin	14.6 µg/L	3.5	16 µg/L	226
Iron	35.09 µmol/L	5.73	37.5 µmol/L	204

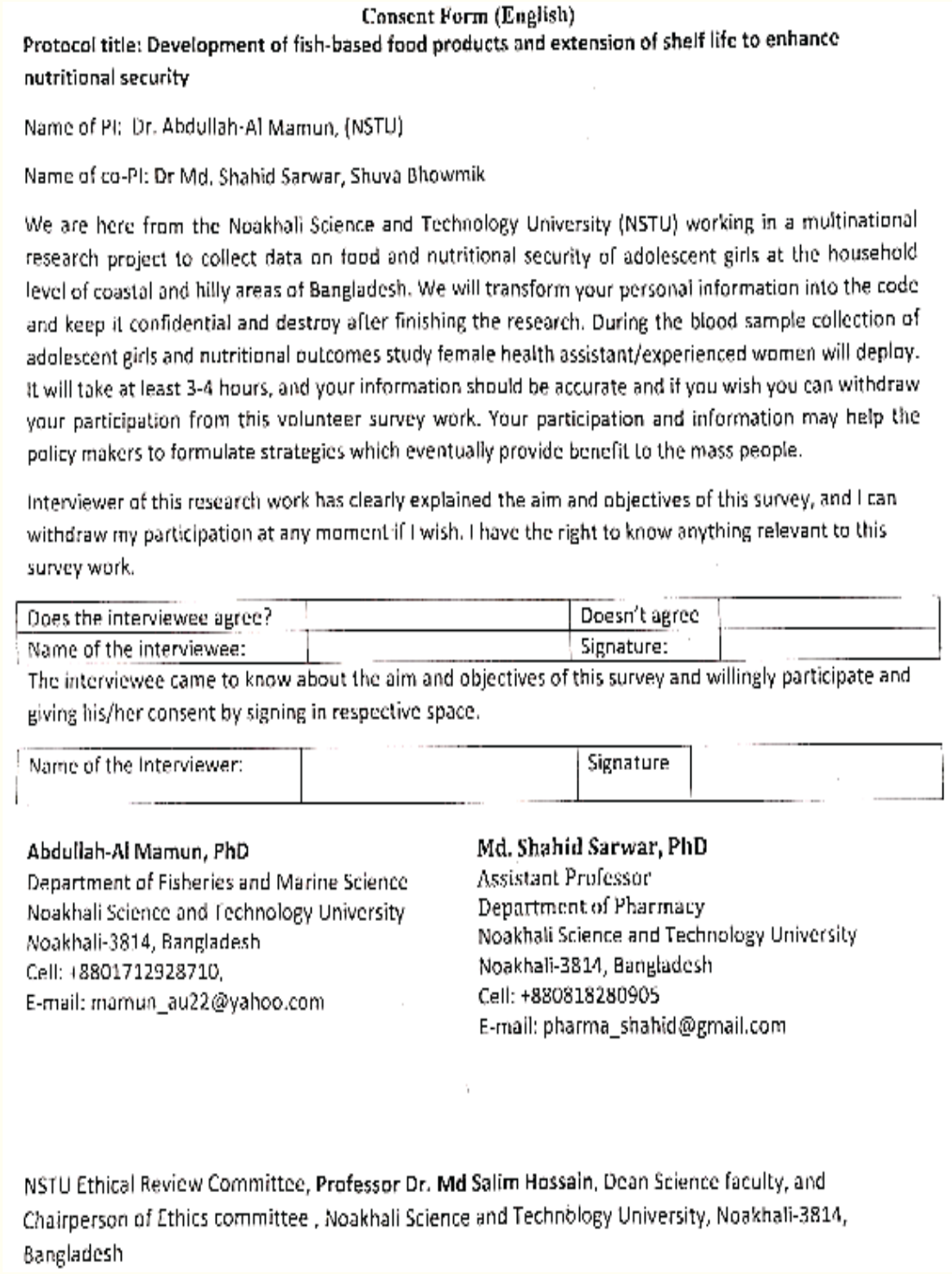
Dose: 40 gm/week/adolescent girl (5.7 gm/day)

Table 4. Recommended daily allowance (%) expected to deliver through Fish Powder supply in the RCT

Biomarkers	RDA	Expected daily intake from powder	% of RDA expected to deliver from fish powder
Vitamin A	700 µg/day	2.052 µg	16.64%
Fe	12 mg/day	1.71 mg	14.25%
Ca	1.1 gm/day	0.24 mg	22%
Vitamin B12	2.4 µg/day	1.6 µg	66.67%

iii. Recruitment, consent, questionnaire, and ethical permission

The data collection team used a screening form to assess initial eligibility of participants. Some participants were excluded at this point (e.g., on the basis of age or unwillingness to participate). Those who were eligible were provided with written and verbal information about the trial. If the participants or their family were willing to continue, they were asked to provide their verbal and signed consent for them and their adolescent girls to participate.



Consent Form (English)

Protocol title: Development of fish-based food products and extension of shelf life to enhance nutritional security

Name of PI: Dr. Abdullah-Al Mamun, (NSTU)

Name of co-PI: Dr Md. Shahid Sarwar, Shuva Bhowmik

We are here from the Noakhali Science and Technology University (NSTU) working in a multinational research project to collect data on food and nutritional security of adolescent girls at the household level of coastal and hilly areas of Bangladesh. We will transform your personal information into the code and keep it confidential and destroy after finishing the research. During the blood sample collection of adolescent girls and nutritional outcomes study female health assistant/experienced women will deploy. It will take at least 3-4 hours, and your information should be accurate and if you wish you can withdraw your participation from this volunteer survey work. Your participation and information may help the policy makers to formulate strategies which eventually provide benefit to the mass people.

Interviewer of this research work has clearly explained the aim and objectives of this survey, and I can withdraw my participation at any moment if I wish. I have the right to know anything relevant to this survey work.

Does the interviewee agree?		Doesn't agree	
Name of the interviewee:		Signature:	

The interviewee came to know about the aim and objectives of this survey and willingly participate and giving his/her consent by signing in respective space.

Name of the Interviewer:		Signature	

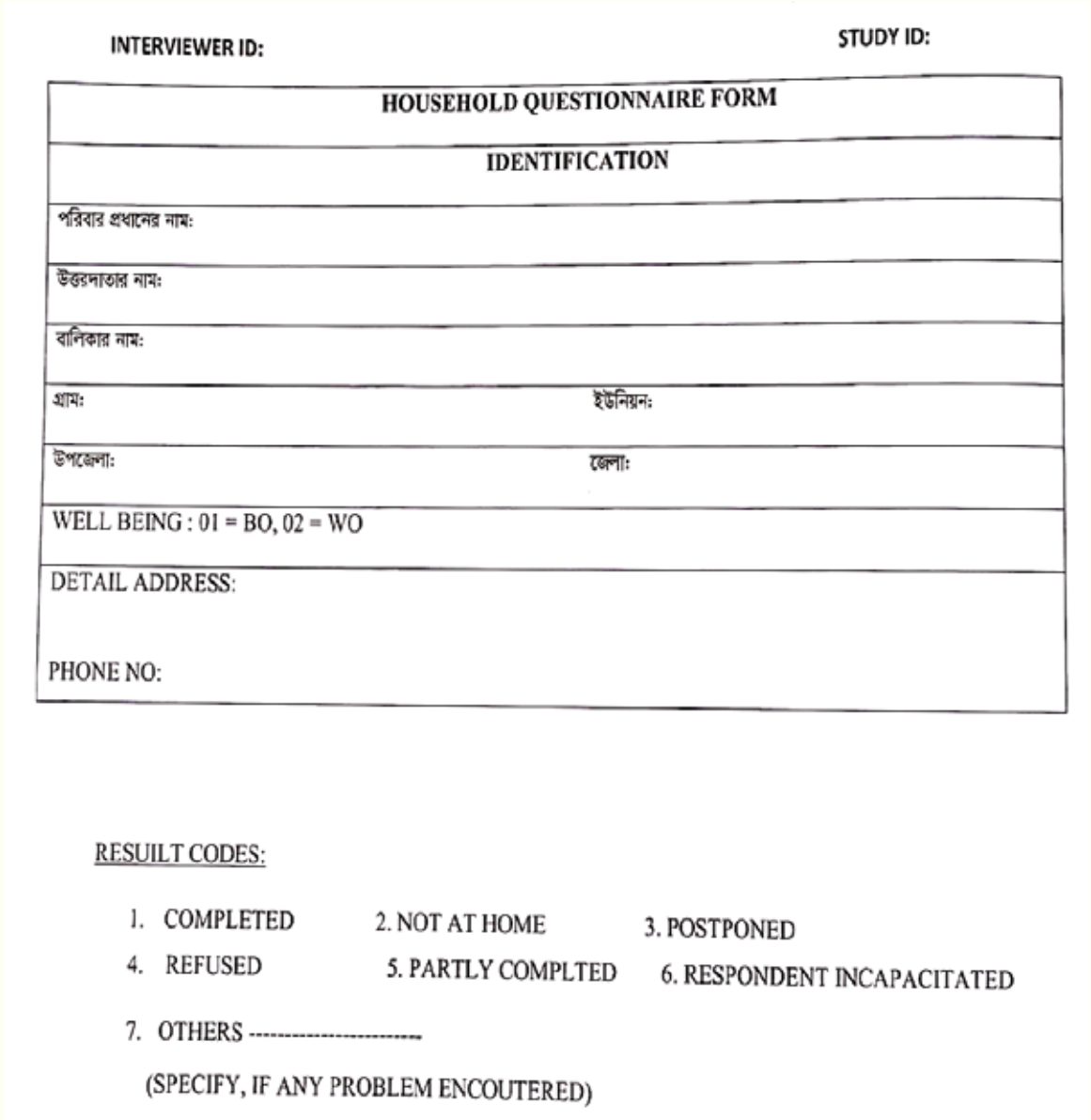
Abdullah-Al Mamun, PhD
Department of Fisheries and Marine Science
Noakhali Science and Technology University
Noakhali-3814, Bangladesh
Cell: +8801712928710,
E-mail: mamun_au22@yahoo.com

Md. Shahid Sarwar, PhD
Assistant Professor
Department of Pharmacy
Noakhali Science and Technology University
Noakhali-3814, Bangladesh
Cell: +880818280905
E-mail: pharma_shahid@gmail.com

NSTU Ethical Review Committee, **Professor Dr. Md Salim Hossain**, Dean Science faculty, and Chairperson of Ethics committee , Noakhali Science and Technology University, Noakhali-3814, Bangladesh

Fig 11. Consent form for the RCT at community level

There was a clear statement that potential participants had the option of not joining the study. If they consented to participate, it was made clear that they could ask questions, make complaints, or withdraw at any time. They have to fill up an approved questionnaire for the purpose of data collection. Ethical permission was taken from the Ethical Committee of Noakhali Science and Technology University.



INTERVIEWER ID: STUDY ID:

HOUSEHOLD QUESTIONNAIRE FORM	
IDENTIFICATION	
পরিবার প্রধানের নাম:	
উত্তরদাতার নাম:	
বালিকার নাম:	
গ্রাম:	ইউনিয়ন:
উপজেলা:	জেলা:
WELL BEING : 01 = BO, 02 = WO	
DETAIL ADDRESS: PHONE NO:	

RESUILT CODES:

1. COMPLETED 2. NOT AT HOME 3. POSTPONED
4. REFUSED 5. PARTLY COMPLTED 6. RESPONDENT INCAPACITATED
7. OTHERS ----------

(SPECIFY, IF ANY PROBLEM ENCOUTERED)

Fig 12. Front page of questionnaire form for the household level survey

iv. Teaming up

The team consist three supervisors including Co-PI Dr. Shahid, Mr. Shuva Bhowmik, one research associate, two researches assistant, one lab attendant, 10 female enumerators and two female health technologists. The team has visited places and conduct research as integral part under the lead of the pI of the component.

Plate 12. Research team including PI, CO-PIs, female enumerators, research associates, and health technologist

v. Training and workshop with intervention HH

Multiple training sessions were conducted among the adolescent girls and their mothers to understand the intervention process of Fish powder and raise the awareness about the nutritional content for future outcomes. A number of trainers from the Core Team appointed for this purpose. Different participatory tools are used and potential feedbacks were recorded during the training sessions.

Plate 13. Training sessions in different locations. A= Presenting Fish powder in a small container, B=Training team with respondents, C=Training with adolescent girls and their mothers and D= Training with mothers of all adolescent girls

Plate 14. Girl with Fish powder recipes preparation leaflet

vi. Household survey

Blood sample collection kits, MUAC tape, height scale (cm), weight scale (for female body weight), measuring cup sets, weight scale (food), food photography album (developed in the training session) was carried out by the researchers during the survey. A person or persons related or unrelated, living together and taking food from the same kitchen considered a household. Consent in writing was obtained from the participants and in some occasion from the legal guardian. Ethical clearance certificate was obtained from the proper authority of NSTU. Food photography was prepared on the cooked major food items to adjust the food weight and portion size (Foster et al., 2006; Nelson et al., 1996). The measuring cup sets were also adjusted with the actual food weights that were also used during the survey. So, these were the combination of taking the direct weight of food, utilised the standardise measuring cup sets, and food photography album used whenever necessary, depending on food type, availability of particular food item during the survey that eaten in last 24 hours. Utilisation of the combination of these three tools was found helpful to extract the actual amount eaten at household level by the individuals. Firstly, family level cooking consumption was measured and then individually asked for their consumption. In the case of children, their parents were given the answer.

vii. Dietary assessment

A 24-hour food recall method and food frequency questionnaire (Fig 12) were developed and validated accordingly. The livelihoods of households, individual diet and nutrition consumption, were sampled on two occasions. All the food items were grouped into ten major food groups based on standard approaches (Handa & Mlay, 2006; INFS, 2013). The major food groups are cereals (boiled rice, puffed rice), vegetables (plants, vegetables, leafy vegetables), pulses (pulses and legumes), fish (seafood, finfish and shellfish), meat (chicken and meat products), milk (milk and milk products), beverage (tea, coffee), eggs, fruits and others (ice-cream, chocolate). To measure food frequency of the main food items consumed over 14 days at household level were recorded in the developed questionnaire. The relationship among the household members was drawn in relation to the household head. Thus, housewife or homemaker was titled as a wife, girls as a daughter, and boys as a son. Although the dietary data of all members of the household were collected only adolescent girl was selected for in-depth dietary analysis.

10.2.1.14 Anthropometric outcomes of adolescent girls

Measurement method like the summation of diet and nutrient intake in association with health outcomes rather than coherent behavioural pattern study are more reproducible and accurate (Tucker, 2010). Anthropometric data (height in cm and weight in kg) were collected by using measuring scale and weight scale. Mid-upper Arm Circumference (MUAC) tape was used to determine the MUAC (in mm) according to Sultana et al. (2015). The adolescent girls were weighed using electronic scales (Tanita Inc. Tokyo, Japan) with a precision of 100 g. Height was measured using locally made standardized wooden length/height boards with a precision of 0.1 cm (1 mm).

10.2.1.15 Blood samples analysis of adolescent girls

Adolescent girls both from control and intervention sites (total 240) were selected for collecting blood samples from adolescent girls (10-19 years old). At least 15% HH was kept on a reserve list for blood samples. In both survey points, blood samples were collected in a health centre, developed as temporary sample collection centers on the same day. To obtain 1200 µl serum, 3.5 ml of venous blood was collected in a Venoject tube. After the blood had been collected from all participants, the blood tube was placed in a cool box and allowed to clot.

The blood samples were kept at room temperature for 30 minutes to clot and centrifuged at 1500 rpm for 15 minutes to collect the supernatant serum. Then serum samples were kept in icebox to store in the refrigerator for determination of iron, ferritin, vitamin A, iodine and calcium. These have been already sent to the respective laboratories in the United Kingdom, South Korea, and ICDDR, B of Bangladesh.

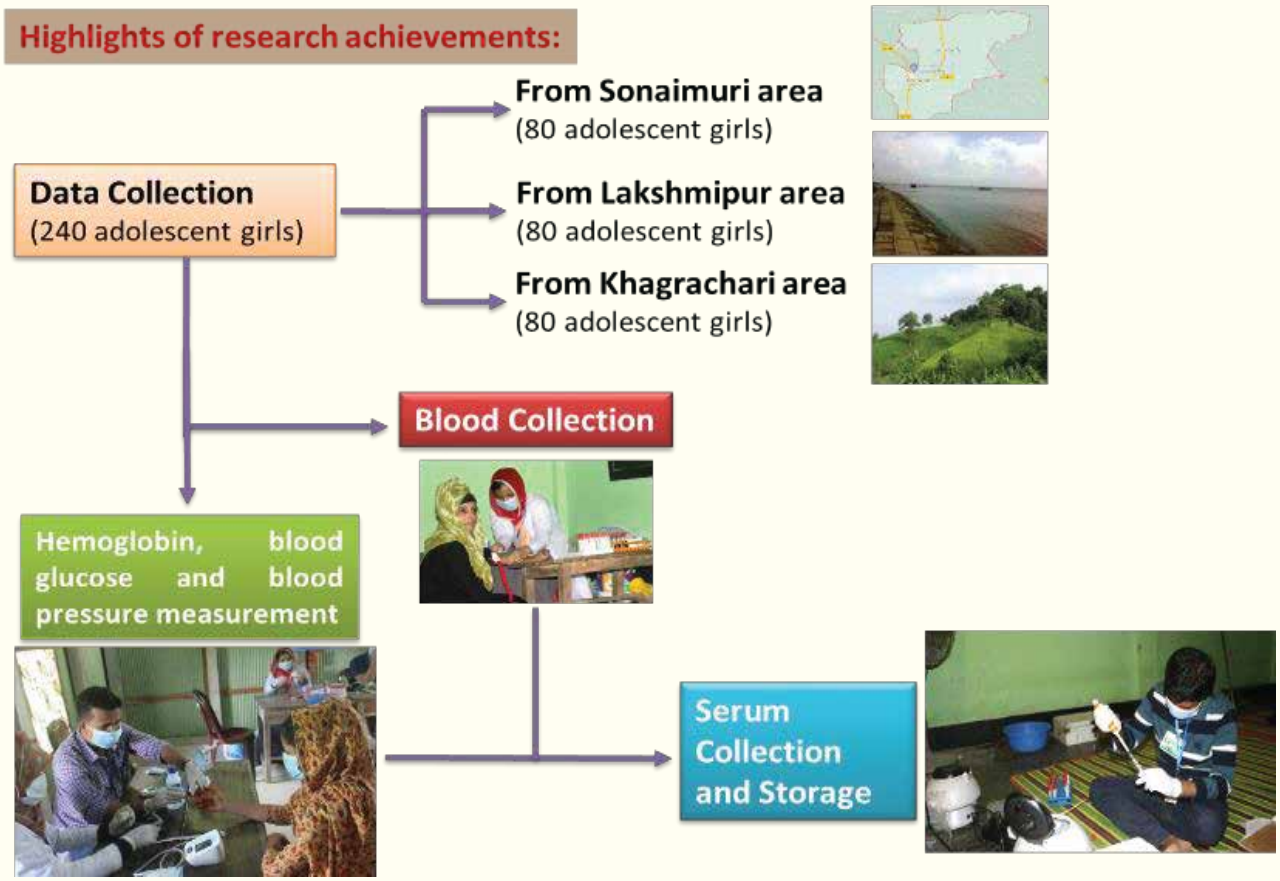


Fig 13. Data collection, blood collection, and storage from different sites

Plate 15. Instruments used in sample collection

At the end of each day, the whole blood was centrifuged and the serum aliquoted into at least three cryovials by pipetting using a disposable pipette. Barcoded labels were provided for each of the study participant's questionnaire forms and each of the aliquoted cryovials. The serum was stored in a freezer (-20ºC or colder) as soon as possible. During the transportation of the serum to the Nutritional Biochemistry Laboratory at icddr, b, the cold chain was maintained. Samples were stored in a -70ºC freezer and analyzed in the Nutritional Biochemistry Laboratory to estimate blood parameters.

10.2.1.16 List of biomarkers and nutrients tested

Blood samples were collected from the target population to measure retinol, CRP, Ca, Fe, Ferritin and vitamin B12. Amount of all micro and macro nutrients, fatty acids, amino acids and vitamin A were measured from produced Fish powder. Besides, iron concentration in drinking water was collected as a supporting data for further analysis.

10.2.1.17 Reference value

Reference values for Body Mass Index (BMI), Mid-Upper Arm Circumference (MUAC), total PUFA (omega-3 and 6) in blood content, micronutrients, protein, and energy were obtained from standard sources; (NIH, 2016; WebMd, 2016). The consumed amount of food item by species and, in some cases, type-wise (tea, ice-cream) were recorded and converted to nutrient content using secondary values (Islam et al., 2010; INFS, 2013). The nutritional data of all food items except fish obtained from secondary sources (Islam et al., 2010; INFS, 2013).

Table 5. Reference values for nutritional study value in parenthesis were considered for the study

Nutrients	Reference value	Reference
BMI	The lower and upper SD boundaries limits -6 to +6	WHO (2011)
MUAC	The lower and upper SD boundaries limits (-6 to +6)	WHO (2011)
Protein	0.83 g/kg body weight	NIH, 2016; WebMd, 2016
Energy	2300 kcal/day	NIH, 2016; WebMd, 2016

Nutrients	Reference value	Reference
LC n-3 PUFA	250-500 (500) mg/day	NIH, 2016; WebMd, 2016
Ca	1300 (1100) mg/day	NIH, 2016; WebMd, 2016; FAO, 2001
Fe	22-62 (9.5) mg/day depended on bioavailability	NIH, 2016; WebMd, 2016; FAO, 2001
Zn	4.6-15.5 (11) mg/day depended on bioavailability	NIH, 2016; WebMd, 2016; FAO, 2001
Se	26-50 (55) µg/day	NIH, 2016; WebMd, 2016; FAO, 2001

Plate 16. Anthropometric data collection and other related activities

10.2.1.18 Water iron content analysis

Tube-well water is mostly consumed by the community people in the study area. Therefore, water was collected at least from 8 wells from each site which in total were 48.

Table 6. Water iron analysis from wells at 6 different locations

SL No	Area	Intervention/Control	No. of tube-well
1	Noakhali	Intervention	8
2	Noakhali	Control	8
3	Lakshmipur	Intervention	8
4	Lakshmipur	Control	8
5	Khagrachari	Intervention	8
6	Khagrachari	Control	8

i. Protocols followed

1. Team used a glass bottle (Polypropylene and Polyethylene can be used) for collecting water samples.
2. Cleansing of the bottles were done as follows:
    - Clean the glassware or plastic ware with laboratory detergent.
    - Rinse well with tap water.
    - Rinse with a 1:1 hydrochloric acid solution or a 1:1 nitric acid solution.
    - Rinse well with deionized water.
    - Air dry the container.
3. Water sample were collected into a large volume of sample in a single container and transfer to smaller bottles. The tube well was pumped for at least for 5 minutes before sampling. The best sampling time is at daytime especially at noon and samples were collected at noon.
4. Before transfer to small bottles add approximately 2.5 mL Nitric Acid 1:1 solution* per 1 L of sample until a pH less than 2 is reached.
5. Use pH indicator paper or a pH meter to make sure that the pH is 2 or less.
6. Such samples were kept in refrigerator at 4^0C before analysis. According to standard data, such samples can be stored for 6 months for determination of iron.

***Nitric Acid 1:1 solution preparation**

Add equal quantity of acid (16M) slowly with equal quantity of water. For example, if you want to prepare 100ml solution add about 50 ml of concentrated acid carefully with 50 ml of distilled water. Keep it for some time to cool and store for further use. The solution must be prepared in glass bottle to avoid risk factor.

List of biomarkers and nutrients tested

a) **From blood serum**
    - ✓ Retinol
    - ✓ CRP
    - ✓ Ca

- ✓ Fe
- ✓ Ferritin
- ✓ Vitamin B12

b) From fish powder

- ✓ All micro and macro nutrients
- ✓ Fatty acids
- ✓ Amino acids
- ✓ Vitamin A

c) From drinking water

- ✓ Iron

10.2.1.19 Intervention period

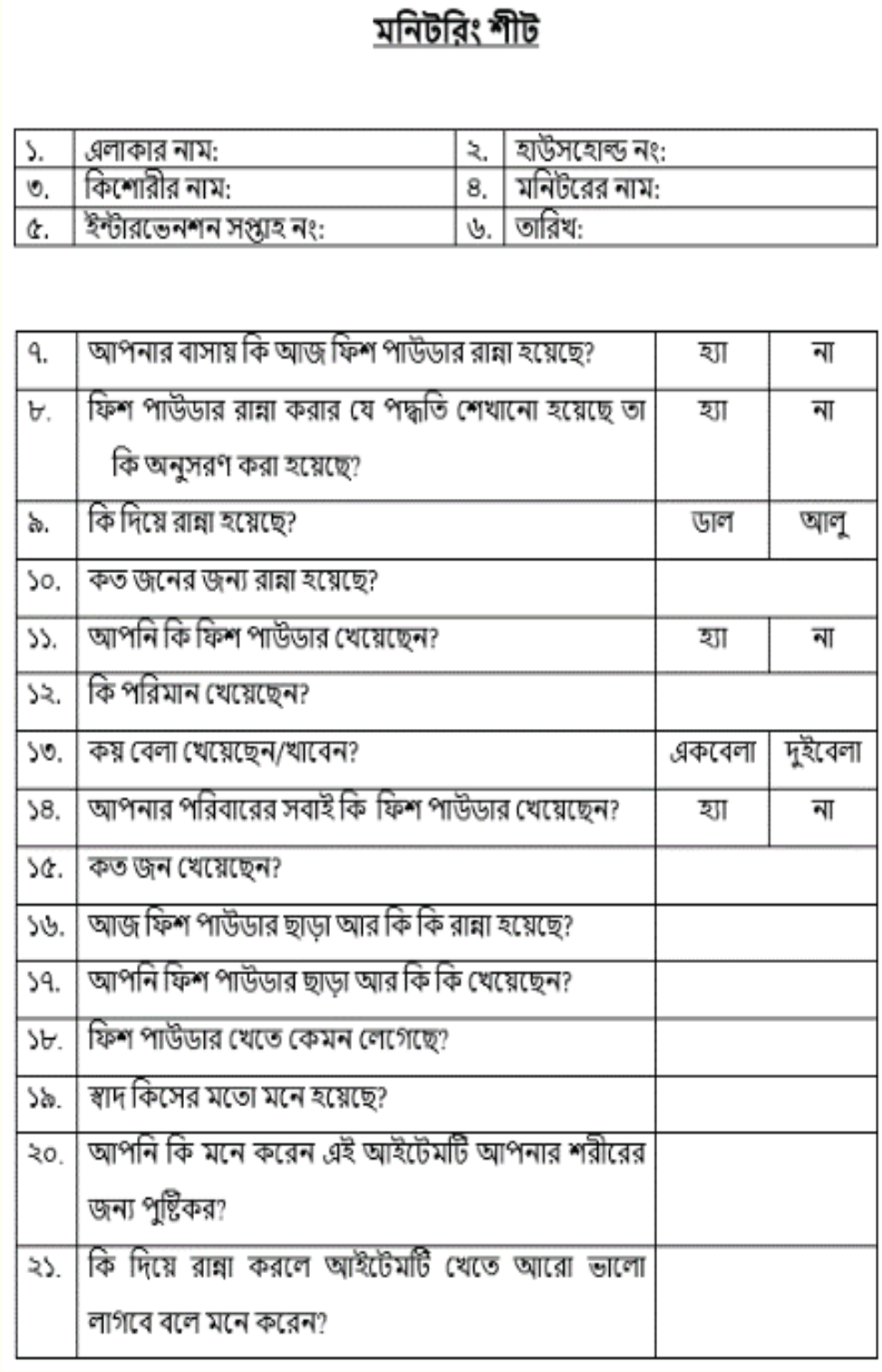
Intervention process started from the third week of October 2020. Participants in the intervention groups were then provided the Fish powder and they continued to consume the Fish powder with their main food for 18 weeks period. Households at intervention sites got hands on training on recipe preparation and also recipe preparation videos were displayed and leaflet were shared with them.

10.2.1.20 Monitor selection

Two female field level monitors were assigned from each intervention site. Monitors were from the same community to communicate with tand visit households every day. The local monitor selection criteria where they must have some previous working experiences in nutrition intervention. Nutrition Training was conducted among the workers for understanding the questionnaires correctly and maintaining that data input device (TAB) in its current form. Hand full documentation with proper guidelines helped this project to gain field data smoothly.

Plate 17. Monitor selection, training, and Tab handover to monitors

A monitoring sheet in local language was prepared where food consumption of adolescent girls is recorded for every day over the intervention period. A TAB (electronic device) was given to local monitor to gather real time information of recipe preparation, consumption and so on.



মনিটরিং শীট

১.	এলাকার নাম:	২.	হাউসহোল্ড নং:
৩.	কিশোরীর নাম:	৪.	মনিটরের নাম:
৫.	ইন্টারভেনশন সপ্তাহ নং:	৬.	তারিখ:

৭.	আপনার বাসায় কি আজ ফিশ পাউডার রান্না হয়েছে?	হ্যা	না
৮.	ফিশ পাউডার রান্না করার যে পদ্ধতি শেখানো হয়েছে তা কি অনুসরণ করা হয়েছে?	হ্যা	না
৯.	কি দিয়ে রান্না হয়েছে?	ডাল	আলু
১০.	কত জনের জন্য রান্না হয়েছে?		
১১.	আপনি কি ফিশ পাউডার খেয়েছেন?	হ্যা	না
১২.	কি পরিমান খেয়েছেন?		
১৩.	কয় বেলা খেয়েছেন/খাবেন?	একবেলা	দুইবেলা
১৪.	আপনার পরিবারের সবাই কি ফিশ পাউডার খেয়েছেন?	হ্যা	না
১৫.	কত জন খেয়েছেন?		
১৬.	আজ ফিশ পাউডার ছাড়া আর কি কি রান্না হয়েছে?		
১৭.	আপনি ফিশ পাউডার ছাড়া আর কি কি খেয়েছেন?		
১৮.	ফিশ পাউডার খেতে কেমন লেগেছে?		
১৯.	স্বাদ কিসের মতো মনে হয়েছে?		
২০.	আপনি কি মনে করেন এই আইটেমটি আপনার শরীরের জন্য পুষ্টিকর?		
২১.	কি দিয়ে রান্না করলে আইটেমটি খেতে আরো ভালো লাগবে বলে মনে করেন?		

Fig 14. Monitoring sheet prepared for data capture form intervention sites

10.2.1.21 Weekly subsidy to HH

To encourage women to lead the program at HH levels. Token money in a weekly basis was given to homemakers. It was useful for the women to buy additional commodities to cook Fish powder. Women feel free to buy required commodities like potato, onion, oil to cook Fish powder-based products.

10.2.1.22 End-line survey

The end line survey was conducted in the same sites and the similar level of data has been collected. In this phase about 10% girls were missing as they got married in the meantime and/or were absent in their household during the survey time. All girls were given a token gift for their participation same as baseline survey. Households at intervention site got token money in each week for their participation during the RCT.

10.2.1.23 In-vivo experiment of Fish powder efficacy

A preclinical trial to evaluate the effect of nutrient-rich fish supplement in anemic mice model.

i. Work plan

The goal of the current study is to determine whether a nutrient-rich fish supplement can reduce anemia. Swiss albino female mice, 35–40 days old, are utilized as test subjects in experiments using the model created by El Kasmi et al. (2012). Initially, body weight, hemoglobin and RBC concentration were determined in all experimental animals. After that mouse were subjected to develop anemia by losing tail blood using blood loss method (Fig 15).

Fig 15. Selected mice for the experimental model and collection of mice serum for further analysis

Work flow

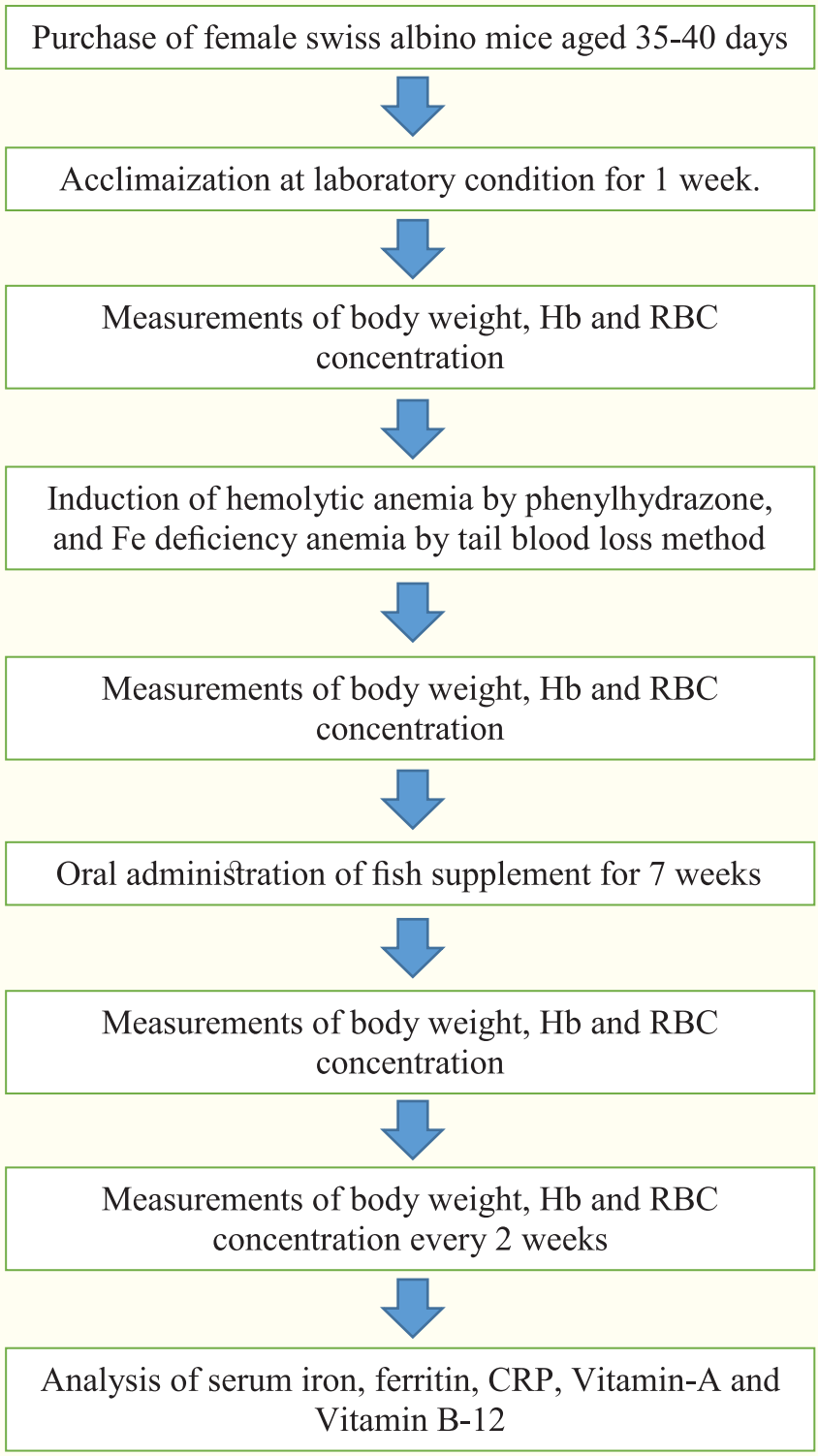


Fig 16. Working steps for the experimental model

Mice were divided into six groups, each group comprising of six mice. They are:

- Group I: Control group of iron-deficiency anemia receiving normal feed
- Group II: Positive control group of iron-deficiency anemia receiving normal feed and multivitamin supplement

- Group III: Iron-deficiency anemia group receiving low dose (10 gm/week) of Fish powder plus normal feed
- Group IV: Iron-deficiency anemia group receiving high dose (20 gm/week) of Fish powder plus normal feed

To confirm the status of anemia; hemoglobin concentration was measured. After successful induction of anemia, we will start oral administration of Fish powder for 4 weeks. Body weight, and hemoglobin concentration were measured every week. After 4 weeks, 100 µL blood was drawn from each mouse and collected in Eppendorf, centrifuged at 1500 rpm for 10 minutes and serum will be collected for analysis.

Plate 18. FP fortified food to mice

Specific amounts of chemicals are

i. Multivitamins
ii. Normal diet
iii. Fish powder

ii. Methodology

At first, female swiss albino mice age 35-40 days were purchased from icddr, b. These mice were kept in laboratory condition for 7 days at ad-libitum. After adaptation, the body weight, hemoglobin level in mice serum was measured. Later these mice were developed iron deficiency anemia by losing tail using blood loss method. When we confirmed anemic condition, we collected blood sample from mice, centrifuge them and later stored the serum for further analysis. Approximately 100 mL serum was collected per mice. This is our baseline data. After serum collection, the intervention period for 28 days started. This time fish powder supplements were given to the mice. After intervention, we again collected blood sample, centrifuged and collected the serum. Then, initial and final serum samples sent to icddr, b for further analysis.

iii. Data analysis

Data were analyzed through one-way ANOVA test. Biomarkers data from mice model were compared with main data of adolescent girl for probable effect and increments.

10.2.1.24. Shelf-life of Fish powder

i. Determination of shelf-life

A shelf-life study is an objective means to determine how long a product can reasonably be expected to keep, without an appreciable change in quality, safety & character.

Accelerated shelf-life studies are a determination process where the trial period is shortened by deliberately increasing the rate of deterioration. This is usually done by increasing the storage temperature.

Plate 19. Experimental instruments for shelf-life test

Plate 20. Tools for shelf-life experiments

ii. Chapala Fish powder collection

Chapila (Gudusia chapra) Fish powder samples packed with zipper and vacuum pack were collected from Department of Fisheries and Marine Science, Noakhali Science and Technology University, Noakhali-3814. Then the samples were kept at 45°C and 55°C for up to 45 days for analysis to predict shelf-life of Ready-to-cook Chapila Fish powder.

iii. Chemical features determination

a. Proximate composition analysis

The moisture content of Chapila Fish powder was determined by evaluating the loss of weight after drying the sample in hot air oven at 105 °C overnight until weight became stable (AOAC, 1990). Ash content of was determined in moisture free dry samples in a muffle furnace at 550 °C for 20 h until all organic components of sample were incinerated completely (AOAC, 1990). Crude protein content of Tilapia samples was determined by multiplying the nitrogen content obtained by Kjeldahl's method by the conversion factor 6.25 (AOAC, 1990). Crude lipid content of tilapia sample was determined by extracting fat using Soxhlet method (AOAC, 1990).

b. Mineral content

To determine the mineral content, Chapila Fish powder samples were dissolved and digested with HNO_3 at 200 °C for 2 h (AOAC, 1995). Phosphorus (P) and iron (Fe) contents in Fish powder sample were determined by atomic absorption spectroscopy method using atomic absorption spectrophotometer model AA-7000 (Gokoglu et al. 2004).

c. pH, TVBN, and peroxide value

pH value of the Chapila Fish powder was determined by using a pH meter (Mettler Toledo 320-s, Shanghi, China) followed standard method (Vyncke, 1981). The TVBN value was measured by using Conway modified micro-diffusion technique (Conway and Byrne, 1933). The peroxide value was calculated according to the American Oils Chemists' Society official method AOCS Cd 8-53 (AOCS, 1998).

d. Fatty acids profile determination

Total lipids and fatty acids are extracted from Fish powder by hydrolytic method. The fatty acids were obtained from saponification of total lipids of Fish powder sample with NaOH and were converted into fatty acid methyl esters (FAMEs) by methylation using HCl and methanol mixture. The composition of fatty acids in Chapila Fish powder was determined by gas chromatographic (GC) method (Kocatepe and Turan, 2012). The comparison of the retention times and peak areas with the respective peak areas of the relevant fatty acids' standard was used to identify and quantify the individual fatty acids (Mohanty et al. 2019).

iv. Shelf-life estimation

The temperature-accelerating factor or temperature quotient, Q_{10} was determined as follows (Perez et al. 2004):

$Q_{10} = (R_2/R_1)^{(10/T_2-T_1)}$…… (i)

Where, R is rate of reaction, T is temperature, and Q_{10} is temperature co-efficient.

In general, Q_{10} value is applied to measure the accelerated aging rate (A) and accelerated aging time duration (B). For typical chemical reactions, Q_{10} values are 2.

$A = Q_{10}^{((Te-Ta)/10)}$…….. (ii)

Where, Ta is ambient temperature, Te is elevated temperature, and Q_{10} is reaction rate.

B = Desired real time/A…… (iii)

10.2.1.25. Consumer preference of Fish powder among urban households in Noakhali

The main objective of the project is to know the consumer preference to use and to buy Fish powder. The survey is very interesting to know the household preference level of Fish powder consumption.

Along with the specific objective the study also covered the following auxiliary objectives:

- To know the consumption habits of Fish powder.
- To know the pattern of using Fish powder with different vegetables.
- To know the influence of using Fish powder in the food security ecology.
- To determine SWOT (strength, weakness, opportunities, and threat) of the Fish powder.

10.2.1.26. Methodology

i. Sample and data collection

The project used survey-based methodology to collect data. We selected Noakhali municipality households for collecting data. The convenience sampling techniques method was used in the survey. Customers were contacted personally. It was directed to the surveyor to provide Fish powder to their familiar family because of the Covid-19 lockdown restriction. To collect data 100 sample product (each sample product contains 250 g Fish power) were distributed to 100 households (minimum 4 member). We have given 10 days of each household to eat this Fish power. After those 100 questionnaires were distributed to those 100 families. Total 401 respondents were found, out of which, 249 responses were completed and valid for the analysis after removing incomplete and unusable response. The respondents were asked to share their views on the taste, effectiveness, usability, benefits and disadvantage of the Fish power.

A total of 144 questions were included in the questionnaires with 6 segments of questions, namely

1) Demographic data
2) Product related data (time + nutrition)
3) SWOT data
4) Item cooked and family preference data
5) Product marketing related data
6) Fish powder safety related data

Plate 21. Data collection through surveying

ii. Data analysis procedure

Data analyzed through Microsoft excel by using graphs, pie charts, column charts, tables and word cloud to get findings of the study.

5.3 Component-2 (RU)

10.3.1 Baseline survey

Baseline data was collected by survey method utilizing structured questionnaire in the superstores of the Dhaka city. Purposive sampling technique was used for selecting four locations of superstores for data collection. The respondents (10) were also selected by purposive sampling method based on availability during the face-to-face interview in different outlets of superstores in Dhaka city (Shwapno, Meena bazaar, Prince Bazar, Nandan etc.). The questionnaire was organized to investigate the superstore managers on the availability of fishery value-added products in the superstores and expected consumers preference on value-added products (Annexure RU:2).

Plate 22. Baseline questionnaire surveys in different superstores in Bangladesh

10.3.2. Experiment-1: Development and quality evaluation of the Ready-to-cook (RTC) and Ready-to-eat (RTE) products

Both marine and freshwater fishes were used for preparation of Ready-to-cook and Ready-to-eat products. For this purpose, freshwater fishes were bought from the farm/market and brought to the Quality Control Laboratory of the Department of Fisheries of the University of Rajshahi in live condition. On the other hand, marine fishes were collected from marine fish landing center in Chattogram or from a commercial fishing vessel as frozen and brought to the Department of Fisheries of Rajshahi University under icing condition. After arriving, freshwater fishes were used directly for product development and marine fishes was kept in frozen storage at -18°C for further processing.

Low-value freshwater and marine fishes were used for product development. Tilapia (*Oreochromis niloticus*) was used for the preparation of Fish ball, Battered & breaded fish, and Fish crackers and thai Pangas (*Pangasianodon hypophthalmus*) was used for the preparation of Fish sausage. On the other hand, skipjack (*Katsuwonus pelamis*) was used for preparation of Fish ball & Fish crackers, Mackerel (*Rastrelliger kanagurta*) was used for preparation of Fish sausage and white Snapper (*Lutjanus johnii*) was used for Battered & breaded fish. In addition, Sardine (*Sardinella fimbriata)* fish was used for the preparation of Fish marinades.

i. Preparation of Fish ball

Ingredients required*

- Corn flour- 40 g
- Ginger paste-10 g
- Garlic paste- 8 g
- Corriander-2.5g
- Cumin-2.5g
- Red chili powder- 3 g
- Black Pepper powder-1.5 g
- Soy sauce- ½ tsp
- Lemon juice- 2 tsp
- Salt- 7.5 g
- Onion-40g
- Green chili-4 pcs
- Bread crumbs- ½ cup

Breaded ingredients**

- Egg-2
- Bread crumbs- ½ cup

The fishes were washed, beheaded and eviscerated. The skinned fishes were filleted and deboned manually under chilled condition. The minces were prepared by a mechanical meat

mincer (Panasonic Corporation, Japan) through a 1mm orifice diameter so that all bones and connective tissues was removed from the muscles.

First 500 g of fish mince was taken in a bowl and then added different ingredients* and mixed well. The mixture was then kept at refrigerator for 30 min. Then made round shaped ball manually. The prepared ball was then soaked in beaten eggs and rolled on bread crumbs**. Fish ball was then ready for MAP packaging and preservation at 4°C.

Plate 23. Preparation of Tilapia Fish ball

ii. Preparation of *Keropok Lekor* type Fish sausage

Ingredients required*

- Corn flour-160 g
- Wheat flour-80g
- Sugar-14g
- Salt- 11g
- Ice-60-100g
- Pepper- 2g
- Chilli powder- 1.5g
- Soya sauce-1/2 tsp

Fish (Pangas/Mackerel) mince was prepared similar to that of Fish ball preparation. First 500 g of fish mince was taken in a bowl and then added different ingredients* and mixed well. The mixture was entered into the sausage stuffer to prepare Fish sausage. Then the sausage was

boiled at hot water until floated. Fish sausage was then ready for MAP packaging and preservation at 4°C.

Plate 24. Preparation of Pangas Fish sausage

iii. Preparation of Battered & breaded fish

Ingredients required*

- Ginger paste-10 g
- Garlic paste- 10 g
- Cumin powder- 2 g
- Coriander powder-2 g
- Red chili powder- 4 g
- Turmeric powder-2 g
- Soy sauce- ½ tbs
- Lemon juice- 2 tsp
- Salt- 7 g

Breaded ingredients**

- Wheat flour- 1 cup with Cumin 2 g, Red chili- 2 g, Salt
- Egg-2
- Bread crumbs-1 cup

Fishes (Tilapia/white Snapper) were filleted first and kept aside. Different ing redients were mixed well in a bowl. The mixture of ingredients was rubbed well on the fillets and kept at the refrigerator for 1h. After that, the fillets were rolled on flour with other ingredients**, soaked in beaten eggs and finally rolled on breadcrumbs. Then the product was kept at refrigerator for 30 min with plastic wrap. Then the Battered and breaded fish fillets were ready for MAP packaging and preservation at 4°C.

Plate 25. Preparation of Battered and Breaded Tilapia fish fillet

Plate 26. Preparation of Battered and breaded white Snapper fish fillet

iv. Preparation of Fish marinades

Soaking Solution*

- Acetic acid-2%
- Salt-10%

Others Ingredients**

- Garlic-7.5g
- Ginger-5 g
- Red chili-2.5 g
- Coriander-1.5 g

- Turmeric- 1.5g and
- Cumin-1 g

Sardine fishes were cut into slices and washed with water. First 500 g of fish slices was taken in a bowl and added 10% salt and 2% acetic acid solutions. Then stored at refrigerator (4°C) for 48 h. The fish slices were taken out and drained off the solution. Then different ingredients* were added and mixed well. Then Fish marinades were ready for MAP packaging and preservation at 4°C.

Plate 27. Preparation of Sardine fish marinades

v. Fish Crackers

vi. Fish crackers

Ingredients*

- Tapioca flour-100 g
- Sugar-6 g
- Salt-5.5 g
- Ice-15 g, and
- Sodium bicarbonate-1 g

Fish (Tilapia/Tuna) mince was prepared similar to that of fish ball preparation. First 90g of fish mince was taken in a bowl and added ingredients* and mixed well. The dough was shaped into cylindrical and steamed for 30 m followed by chilling in refrigerator overnight. On the next day, cylinder shaped product was cut into 2 mm thickness and put into oven of 50°C for 10 to 12 hours until the moisture content reading was 10% ± 2%. Dried crackers were then deep fried in vegetable oil at 170-180°C. Then the Fish crackers were ready as ready-to-eat (RTE) products for MAP packaging and preservation at room temperature.

Plate 28. Preparation of Tilapia Fish Crackers

Plate 29. Preparation of Tuna Fish crackers

The quality of the RTC and RTE fish products was evaluated by the following biochemical and sensory quality:

Proximate composition

The proximate composition (moisture, protein, lipid, ash) analysis were done as per standard protocol using AOAC method (AOAC, 1995).

Stuctural test

The structure of the fish products was done by standard method using a texture analyser (FRTS-100, Japan).

Color test

Color test including L*, a*, b* values were performed by the standard method using a colorimeter Colorimeter (NH310, China).

Sensory evaluation

Sensory characteristics of the RTC and RTE products was evaluated by selected panel members of the department who have experience in evaluation of similar products, on a nine-point scale and scores were assigned with '1' being the least and '9' being the highest for attributes as described by Reddy et. al. (1992).

10.3.3. Experiment-2: The overall quality and shelf-life of Ready-to-cook (RTC) and Ready-to-eat (RTE) fish products under modified atmosphere packaging at different storage temperature

10.3.3.1. Packaging and storage of RTC products

The RTC fish products (Fish ball, Battered & breaded fish, Fish marinades and Fish sausage) were packed in tray with plastic wrap. The following criteria were considered in selecting packaging material for MAP foods viz. resistance to puncture, sealing ability, antifogging properties, low carbon dioxide permeability, low oxygen permeability, low water transmission rate. In this case, for top wrapping, a multilayered plastic pouch (PE/PA/PE) was used. For tray, thermoformed trays made of HIPS or PVC are ideal for modified atmosphere packaging (Gopal and Shankar, 2001). In all cases, four types of packaging were applied under modified atmosphere packaging with different gas ratio using the method described by Noseda et al. (2012) with some modifications using a tray sealer packaging unit (Multivac, Haggenmueller, Germany) attached with Gas Mixer KM100-3 MEM (WITT, Germany). Analysis of the O_2, N_2 and CO_2 levels in the headspace of the packaged samples were performed with Oxybaby M+ gas analyzer (WITT, Germany). Those four types of packaging were used as treatments namely, (a) aerobic, not sealed as control; (b) MAP 1 (50%CO_2 & 50%N_2) as treatment-1; (c) MAP 2 (40% CO_2, 30% N_2 & 30% O_2) at treatment-2 and (d) MAP 3 (50% CO_2 & 50% O_2) as treatment-3. The packaged products were stored at 4˚C. The fish products in triplicate were subjected to biochemical and microbiological analysis in 4-10 days interval based on products during the storage period at 4˚C in the laboratory.

10.3.3.2. Packaging and storage of RTE products

RTE fish products were packed in plastic film performed by same way used for RTC products. Four types of packaging were used as treatments namely, (a) air pack as control; (b) MAP 1 (100% N_2) as treatment-1; and (c) MAP 2 (50% CO_2 & 50%N_2) at treatment-2. The RTE

products in triplicate were subjected to biochemical and microbiological analysis at a month interval during the storage period at room temperature in the laboratory.

10.3.3.3 Biochemical and microbiological analysis

pH : Ten gram of fish flesh was homogenized with 50 mL of distilled water and the pH value of the homogenate was measured by means of a glass electrode pH meter (Hanna Inst, USA).

Plate 30. MAP packaging of RTC and RTE products

Free Fatty Acids (FFA): The percentage of the free fatty acid (FFA) was calculated for the fish samples using AOAC (1980). 1.0 g of fish sample was measured into 250 mL flask. 25 mL of alcohol and 1.0 mL of phenolphthalein indicator was added to it. The mixture was titrated with aqueous 0.5 N NaOH used in the titration corresponded to the percentage of free fatty acid calculated using the following equation.

$$\% FFA = \frac{Vol\ of\ the\ titrant(mL)\ x\ Normality\ of\ NaOH\ x\ 5.61}{Wt\ of\ sample\ used}$$

The FFA figure was calculated as oleic acid (1mL of 0.1M sodium hydroxide = 0.0282g oleic acid).

Total volatile base nitrogen (TVB-N): Total volatile base nitrogen (TVB-N) was determined according to EC (2005) method. The value-added products from each of the pack was ground carefully using a blender. Exactly 10 g of the ground fish sample was weighed out into a suitable container and mixed with 90.0 mL of 6% perchloric acid solution, homogenised for two minutes with a blender (Bajaj, India), and then filtered. Fifty (50) mL of extract obtained with 4-6 drops phenolphthalein indicator was put in a Kjeldahl flask. After adding some glass-beads,

6.5 mL of 20% NaOH or more was added as per requirement to the flask after placing on the distillation unit and steam distillation was started immediately.

The steam distillation was regulated so that around 100 mL of distillate is produced in 10 minutes. The distillation outflow tube was submerged in a receiver with 100 mL 3% boric acid solution, to which three to five drops of the mixed indicator solution (2 g methyl-red and 1 g methylene-blue was dissolved in 1000 mL 95 % ethanol) was added. After around 10 minutes, distillation was ended. Then distillation outflow tube was removed from the receiver and washed out with water. The volatile bases contained in the receiver solution was determined by titration with 0.01(N) hydrochloric acid solution and regaining the violet colour of mixed indicator confirmed the end-point. A blank test was carried out by the same procedure without using sample. The TVB-N concentration is calculated using the following equation:

$$\text{TVB} - \text{N (mg/100 g sample)} = \frac{(V_1 - V_0) \times 0.14 \times 2 \times 100}{M}$$

V1 = Volume of 0.01 (N) HCl in mL for sample
V0 = Volume of 0.01 (N) HCl in mL for blank
M = Weight of sample in g.

Thiobarbituric acid reactive substances (TBARS): TBARS values were measured using the procedure of Park et al. (2007). Twenty grams of fish muscle was homogenized with 50 mL of 20% trichloroacetic acid (in 2 M phosphoric acid) at 10000 × g for 2 min using a homogenizer (T18, IKA, Germany). The resulting slurry was then transferred into a 100 mL mass cylinder. The slurry was diluted to 100 mL with dd-water and homogenized. Later approximately 50 mL was filtered through filter paper (Whatman No. 1, 110 mm), 5 mL of filtrate was transferred into a test tube and 5 mL of 2-thiobarbituric acid (0.005 M in dd-water) was added. The test tube was shaken well and kept in the dark for 15 h at room temperature. The reactive substances were measured at 530 nm using a spectrophotometer (UV-Visible Spectrophotometer, UV-1601PC, Shimadzu, Japan). Two replicates of 20 g sample were taken for the measurement. TBARS values was calculated as follows:
TBARS value (mg malonaldehyde/kg) = optical density (O.D.) × 5.2

The aerobic plate count (APC): APC was enumerated using plate count agar by APHA (1992) method. Twenty-five grams of fish was aseptically weighed and homogenized with 225 peptone physiological saline (PPS) for 30 sec in a stomacher blender. The homogenized sample was serially diluted using 9 mL PPS for bacteriological analysis. The aerobic plate count was determined by using pour plate method by incubating at 35°C for 24h.

Statistical Analysis: The values were expressed as mean ± standard deviation. Differences among treatments was estimated by using one-way ANOVA with the application of Tukey test using SPSS Version-20, where p values <0.05 were considered as significant.

10.3.4. Experiment-3: Consumer's acceptability of RTC and RTE fishery products in the superstores of Dhaka city

Consumer's acceptability and willingness to buy the packaged RTC and RTE fish products were investigated by survey method utilizing a structured questionnaire (Annexure RU:2). Purposive sampling technique was used for selecting three locations of stores in Amana big Bazar, Rajshahi; RU souvenir shop, RU campus; BARC canteen, Farmgate, Dhaka for data collection. Then, prepared and packaged RTC and RTE fish products were displayed in three retail stores. Consumer's acceptability of the supplied modified atmosphere packaged RTC and RTE fishery products was investigated by survey method utilizing structured questionnaire. The number of respondents (50), selected by voluntary response sampling method from each of the stores were surveyed. The fill-up questionnaires were collected by post, courier, email, WhatsApp and directly etc after eating the purchased products at home. The questionnaire was developed, pre-tested in field situation and necessary modifications were made prior to final data collection. The questionnaire was organized to investigate the consumer's socio-economic condition and preferences and acceptability of MAP packaged RTC and RTE fishery products on different aspects including organoleptic characteristics, price, pack size, quality and overall attraction of the products.

Statistical analysis: For the survey method, all the surveyed data was tabulated and subjected to descriptive analyses by using SPSS Version-20.

Plate 31. Product marketing and consumers acceptability study at different outlets

11. Results and discussion

11.1.Component 1 (NSTU)

11.1.1. Nutrient content of Fish powder

Protocol: After a series of trail the following protocol was developed for fish powder production.

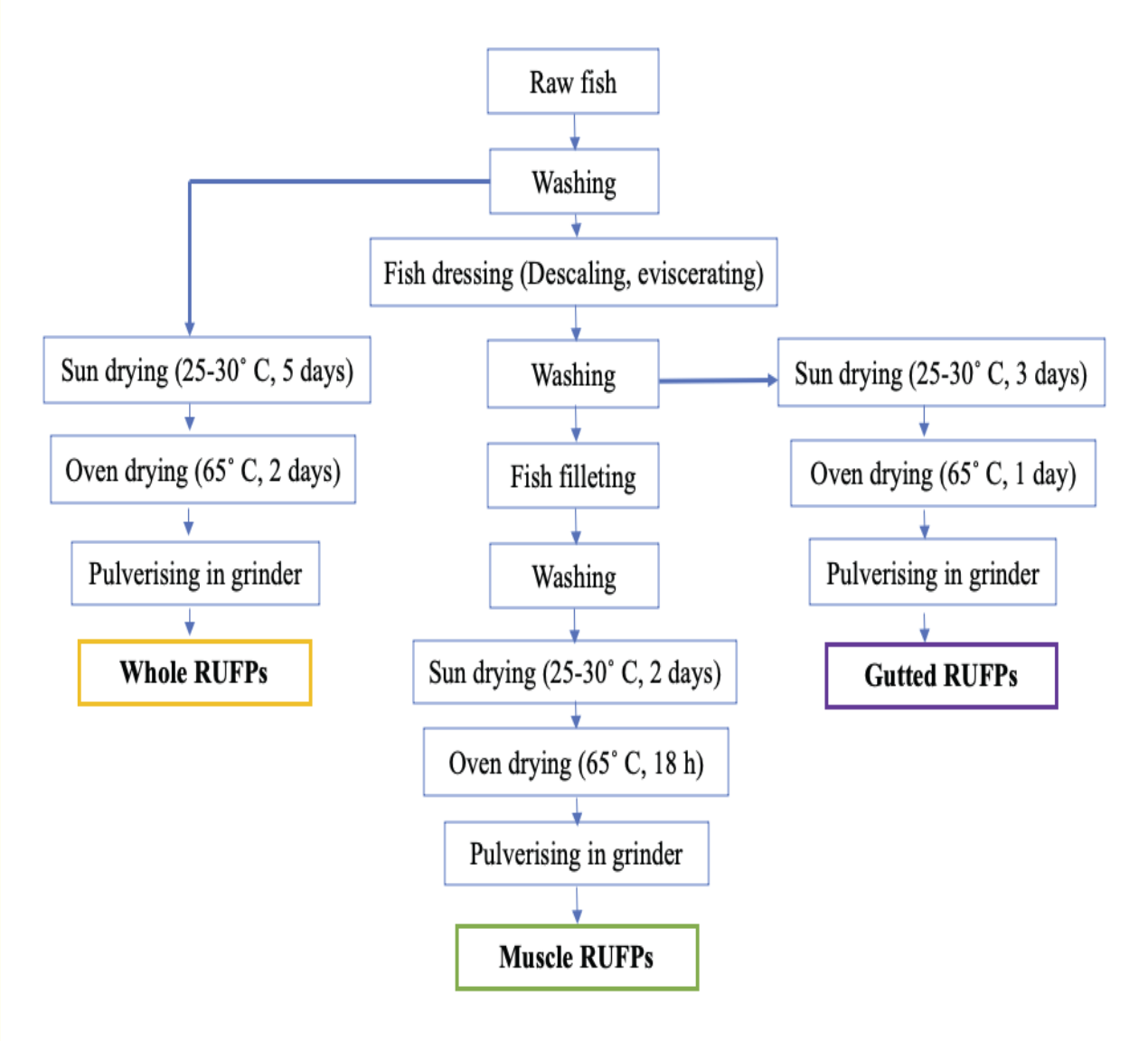


Fig 17. A schematic diagram of Ready to use fish products (RUFPs) preparation from whole, gutted and muscle portions of three fresh water (Punti, Chapila and Kachki) and two marine (Anchovy and Sardine) small fish species in Bangladesh.

i. Weight loss of raw fish in the different RUFP development phases

The percentage of weight loss during the powder preparation process from Chapila, Punti, and Kachki is presented in Table 7. The whole fish yielded the most powder while the muscle yielded the least powder. The whole fish was used immediately after washing and therefore found the weight intact; however, the weight was decreased in gutted fish (12.33–18.8%) and fish muscle (36.67–50%) (Table 7**).** All parts of the fish were used in the whole Fish powder whereas only the muscle portion was used in the muscle powder. On the other hand, scales, gills, fins, and viscera were discarded from all fish during the preparation of gutted Fish powder. Hence, the whole fish produced the highest amount of powder, followed by gutted fish and muscle. The raw weight was also lost due to drying and processing. Among the powders, the whole anchovy fish produced the highest amount (190.5±12.76 g or 19.05%) and the punti muscle produced the lowest amount of powder (53.5 ± 6.73 g or 5.35%) (Table 7**)**. Regarding the quantitative yield among the five species, anchovy produced the highest amount of powder (19.05%) followed by Sardine (15.25%), Chapila (13.65%), Kachki (12.81%), and punti (11.25%).

Table 7. The volume of fish loss in g (±SD) and percentage in parenthesis during the preparation of RUFPs in Bangladesh

Phases	chapila			punti			Kachki	Anchovy			Sardine		
	Whole	Gutted	Muscle	Whole	Gutted	Muscle	Whole	Whole	Gutted	Muscle	Whole	Gutted	Muscle
Raw fish weight (g)	1000 (100%)	876.67±18.86 (87.67%)	633.33±15.15 (63.33%)	1000 (100%)	844.5±17.51 (84.45%)	500.07±14.74 (50%)	1000 (100%)	1000±14.67 (100%)	818±25.43 (81.8%)	525±18.57 (52.5%)	1000±17.44 (100%)	837.5±22.98 (83.7%)	490±13.24 (49%)
Sun-dried weight of fish (g)	195.5±16.35 (19.55%)	132.5±15.11 (13.25%)	92.9±10.57 (9.29%)	185±14.67 (18.5%)	103.2±11.32 (10.32%)	72.7±9.27 (7.27%)	197.14±12.14 (19.71%)	230.5±16.33 (23.05%)	182±18.21 (18.2%)	135.5± 9.34 (13.5%)	248.5±27.34 (24.85%)	162.5±12.65 (16.2%)	107±10.76 (10.7%)
Ready to use (g)	136.5±11.19 (13.65%)	101.5±10.97 (10.15%)	70.4±6.33 (7.04%)	112.5±9.43 (11.25%)	77.6±8.08 (7.76%)	53.5±6.73 (5.35%)	128.06±8.54 (12.81%)	190.5±12.76 (19.05%)	142.5±19.61 (14.2%)	116±13.87 (11.6%)	152.5±11.54 (15.25%)	106±15.09 (10.6%)	85.5±8.71 (8.5%)

ii. Proximate composition of RUFPs

The proximate composition (moisture, protein, lipid, ash, and carbohydrate) of RUFPs developed from five low-cost fish species are presented as the mean ± SD (%) in Table 8 and 9. The moisture content was highest in the RUFPs developed from whole fish, followed by gutted fish and the muscle of fish. The moisture content was highest (11.02 ± 0.07%) in the whole Punti powder and lowest (6.63±0.09 %) in the Anchovy muscle powder. The moisture content in all developed powders was found lower than the standard level which hinders the growth of microorganisms and makes the product stable. The protein content was found to be significantly higher in the powder developed from the muscle of fish than that of gutted and whole fish. The protein content was found highest in the Anchovy muscle powder (72.55±0.49) and lowest in the whole Punti powder (48.81±0.40). Among all the developed RUFPs, the lipid content varied from 6.42±0.06% to 17.58 ± 0.10%. The highest lipid content was in the whole Punti powder (17.58 ± 0.10%) and the lowest lipid content was in the Anchovy muscle powder (6.42±0.06%). The amount of ash was significantly higher in the RUFPs from whole fish compared with the powders from gutted fish and muscle. The presence of bones, viscera, and fins as edible portions is responsible for the higher ash concentration in the RUFPs developed from whole fish. The highest amount of carbohydrate was determined in the whole Sardine powder (9.19±0.07%) and the lowest was in the whole Chapila powder (2.54 ± 0.04%). So, RUFPs of these small fish species have the potential to produce nutrient-dense food, particularly for children, adolescent girls, adults, and pregnant women. When practically all factors are considered, the muscle powder of all of these fish species can can play a vital role for customers those suffer from protein-energy malnutrition or who does not make the effort to acquire expensive protein-rich foods. In contrast, for individuals who can meet their daily protein needs but cannot satisfy their fat and micronutrient requirements, whole Fish powder from any of these species can be extremely beneficial

Table 8. Proximate composition (% ±SD) of RUFPs developed from low-valued Chapila, Punti, and Kachki fish in Bangladesh

Types	Chapila			Punti			Kachki
	Whole	Gutted	Muscle	Whole	Gutted	Muscle	Whole
Moisture	10.38±0.07^{b}	9.86±0.08^{d}	8.38±0.07^{f}	11.02±0.07^{a}	10.07±0.06^{c}	9.41±0.08^{e}	9.90±0.09^{d}
Protein	53.60±0.40^{f}	65.28±0.53^{b}	69.61±0.22^{a}	48.81±0.40^{g}	55.63±0.40^{e}	62.15±0.47^{c}	57.65±0.08^{d}
Lipid	12.63±0.09^{d}	8.45±0.08^{f}	6.86±0.05^{g}	17.58±0.10^{a}	15.14±0.06^{b}	11.43±0.06^{e}	14.28±0.06^{c}
Ash	21.05±0.04^{a}	13.02±0.07^{d}	10.28±0.07^{f}	19.51±0.06^{b}	13.31±0.05^{c}	9.99±0.05^{g}	11.88±0.08^{e}
Carbohydrate	2.54±0.04^{f}	3.43±0.06^{e}	4.84±0.04^{c}	3.94±0.05^{d}	4.75±0.07^{c}	6.19±0.08^{b}	6.30±0.07^{a}

Values are mean ± SD, and values within the same rows with different superscripts are statistically different at $P < 0.05$.

Table 9. Macronutrient's composition (% ±SD) of Ready-to-use food products (RUFPs; Fish powder) developed from sundried Anchovy and Sardine fish in Bangladesh

Types	Anchovy			Sardine		
	Whole	Gutted	Muscle	Whole	Gutted	Muscle
Moisture	9.04±0.14^{e}	7.96±0.08^{d}	6.63±0.09^{a}	10.32±0.09^{f}	8.19±0.06^{c}	7.44±0.07^{b}
Protein	57.38±0.45^{f}	65.27±0.49^{d}	72.55±0.49^{a}	53.52±0.42^{e}	61.56±0.41^{c}	69.59±0.39^{b}
Lipid	11.66±0.056^{a}	8.76±0.07^{c}	6.42±0.06^{f}	12.24±0.10^{b}	10.16±0.07^{e}	8.58±0.05^{d}
Ash	14.09±0.07^{b}	10.74±0.06^{c}	8.67±0.06^{e}	14.31±0.07^{a}	12.09±0.04^{d}	8.00±0.06^{f}
Carbohydrate	7.27±0.04^{b}	6.47±0.06^{d}	4.43±0.06^{c}	9.19±0.07^{f}	7.06±0.06^{a}	6.32±0.06^{e}

Values are mean ± SD, values within same rows with different superscripts are statistically different at $P < 0.05$.

iii. Mineral contents of RUFPs

Minerals are vital elements for maintaining homeostasis inside the human body, although they are required in very trace amounts. Macro-minerals, micro-minerals, and ultra-trace minerals were detected in the RUFPs developed from Chapila, Punti, Kachki; Anchovy and Sardine are presented as mg/kg in Table 10 and 11. The sodium content was highest in the gutted Anchovy powder (4998.73±18.82 mg/kg) and lowest in the Chapila muscle powder (1859 ± 27.81 mg/kg). The whole Anchovy powder had highest amount of magnesium (2364.46±9.53 mg/kg) while the Sardine muscle powder had the lowest magnesium content (1272.26±1.34 mg/kg). The whole Anchovy Fish powder had the highest amount of calcium (61808.9±24.94 mg/kg) while the Sardine muscle powder had the lowest amount of calcium (17805.61±103.89 mg/kg). The study revealed that the sodium-to-potassium (Na/K) ratio was < 1 in all the developed Fish powders. But, Yeom et. al. (2012) pointed out that a Na/K ratio > 1 in any food material may be harmful to the health of consumers. So, the RUFPs developed in this study are safe for human consumption. The highest amount of chromium was found in the whole Sardine powder (10.06±0.20 mg/kg) and the lowest was found in whole Kachki powder (0.30 ± 0.03 mg/kg). The highest level of zinc was found in the whole Kachki Fish powder (128.25 ± 0.69 mg/kg) and lowest in the Anchovy muscle powder (66.01±1.04 mg/kg). However, in our present study, we found all these heavy metals (Cd, As, Pb, Cr, Ni, Zn, Se, Mn, V, Cu, Mn, Fe and Co) were below the recommended tolerable limit set by FAO/WHO [33]. In addition, based on Bogard et al. [34] 15 g/day, 12 g/day and 8 g/day of Fish powder are recommended for pregnant and lactating women, adults and infants, respectively. Consequently, the developed Fish powders are safe for human consumption.

Table 10. Mineral composition (mg/kg±SD) of RUFPs from Chapila, Punti and Kachki fish in Bangladesh

Minerals	Chapila			Punti			Kachki
	Whole	Gutted	Muscle	Whole	Gutted	Muscle	Whole
Macro minerals							
Sodium (Na)	2077.72±5.09^{c}	2134.10±1.73^{b}	1859±27.81^{e}	2060.51±8.33^{c}	2224.98±20.23^{a}	2045.09±3.55^{c}	1984.17±50.41^{d}
Magnesium (Mg)	1882.76±4.17^{a}	1740.53±4.89^{b}	1580.93±1.26^{c}	1498.93±12.98^{d}	1432.94±6.79^{e}	1334.41±6.24^{f}	1856.3±37^{a}
Phosphorus (P)	26041.01±358.14^{a}	21475.27±94.22^{b}	13379.05±37.03^{e}	20922.19±98.64^{c}	21507.96±231.28^{b}	16434.82±33.34^{d}	21325.4±427.49^{bc}
Potassium (K)	2686.46±10.2^{f}	2958.04±22.64^{e}	2210.32±34.72^{g}	6218.38±4.98^{c}	7585.00±57.69^{b}	8512.14±10.44^{a}	5188.75±36.87^{d}
Calcium (Ca)	51909±263.95^{a}	42389.03±161.07^{b}	25091.4±59.88^{f}	38915.6±202.59^{c}	38315.11±251.62^{c}	26381.57±431.12^{e}	35969.95±684.09^{d}
Na/K	0.77	0.72	0.84	0.33	0.29	0.24	0.38
Micro minerals							
Chromium (Cr)	2.55±0.005^{b}	1.12±0.03^{c}	0.9±0.01^{d}	4.16±0.03^{a}	0.83±0.015^{d}	0.86±0.14^{d}	0.30±0.03^{e}
Manganese (Mn)	4.18±0.49^{a}	3.99±0.26^{b}	2.19±1.38^{c}	3.41±0.35^{e}	3.13±0.13^{f}	2.01±0.36^{g}	4.08±1.55^{d}
Iron (Fe)	430.66±24.96^{a}	318.12±3.34^{d}	131.65±0.93^{c}	328.02±0.36^{b}	188.06±3.66^{f}	132.57±3.53^{g}	215.88±13.24^{e}
Cobalt (Co)	0.30±0.01^{a}	0.17±0.005^{d}	0.2±0^{c}	0.29±0^{b}	0.1±0^{e}	0.08±0.005^{f}	0.09±0.005^{e}
Copper (Cu)	4.45±0.01^{c}	4.32±0.19^{c}	5.05±0.19^{b}	5.33±0.17^{a}	3.50±0.11^{e}	3.55±0^{e}	3.96±0.22^{d}
Zinc (Zn)	119.78±0.11^{f}	109.25±2.59^{d}	105.41±1.53^{g}	127.41±0.18^{e}	125.39±0.005^{b}	117.24±0.47^{c}	128.25±0.69^{a}
Selenium (Se)	1.08±0.03^{c}	1.31±0.02^{b}	0.81±0.01^{f}	0.91±0.01^{e}	0.96±0.005^{d}	0.95±0.02^{d}	1.48±0.03^{a}
Ultra-trace Minerals							
Arsenic (As)	1.58±0.01^{b}	1.36±0.07^{c}	2.43±0.05^{a}	0.79±0.03^{d}	0.69±0.005^{e}	0.59±0.005^{f}	1.30±0.02^{c}
Nickel (Ni)	1.73±0.06^{b}	0.82±0.05^{c}	0.74±0.16^{c}	2.4±0.11^{a}	0.41±0.02^{d}	0.50±0.05^{d}	0.23±0.06^{e}
Vanadium (V)	1.05±0.02^{a}	0.71±0.02^{d}	0.77±0.005^{c}	0.95±0.02^{b}	0.5±0.01^{e}	0.27±0^{f}	0.25±0.01^{f}
Cadmium (Cd)	0.03±0.005^{b}	0.05±0.005^{b}	0.03±0.01^{b}	0.25±0.21^{a}	0.02±0^{b}	0.02±0.01^{b}	0.05±0.02^{b}
Lead (Pb)	0.96±0.06^{a}	0.73±0.09^{b}	0.57±0.05^{c}	0.47±0.01^{dc}	0.47±0.01^{dc}	0.55±0.01^{c}	0.40±0.09^{d}

Values are mean ± SD, values within same rows with different superscripts are statistically different at $P < 0.05$.

Table 11. Mineral's composition (mg/kg±SD) of Ready-to-use fish products (RUFPs; Fish powder) developed from sundried Anchovy and Sardine fish in Bangladesh

Minerals	Anchovy			Sardine		
	Whole	Gutted	Muscle	Whole	Gutted	Muscle
Essential minerals (Macro-nutrients)						
Sodium (Na)	4515.91±33.22^{c}	4998.73±18.82^{a}	4394.89±27.44^{b}	3163.21±2.84^{e}	3449.04±123.7^{d}	3146.49±8.73^{e}

Magnesium (Mg)	2364.46±9.53^{a}	2061.37±2.12^{b}	2005.94±7.87^{c}	1888.5±1.11^{d}	1292.29±55.31^{e}	1272.26±1.34^{e}
Phosphorus (P)	25607.54±41.08^{a}	18544.01±23.32^{d}	14506.91±160.16^{e}	23042.19±65.01^{b}	19633.88±50.8^{c}	13363.53±78^{f}
Potassium (K)	4837.44±43.03^{e}	5593.98±16.96^{d}	6353.21±8.69^{d}	5060.08±1.73^{c}	7008.43±85.25^{b}	7262.22±13.92^{a}
Calcium (Ca)	61808.9±24.94^{a}	40522.63±260.10^{c}	25326.71±505.09^{e}	42035.23±44.21^{b}	31068.42±1034.61^{d}	17805.61±103.89^{f}
Na/K	0.93	0.89	0.69	0.63	0.49	0.43
Trace-elements (Micro-nutrients)						
Chromium (Cr)	2.49±0.09^{c}	3.35±0.19^{b}	0.63±0.02^{f}	10.06±0.20^{a}	1.06±0.22^{e}	1.57±0.02^{d}
Manganese (Mn)	16.17±0.46^{d}	11.72±0.16^{e}	10.7±0.17^{f}	64.05±0.005^{a}	25.63±1.13^{b}	18.92±0.12^{c}
Iron (Fe)	118.26±3.04^{d}	84.28±0.16^{e}	77.75±0.28^{e}	232.42±9.31^{a}	216.38±25.03^{c}	171.39±9.24^{b}
Cobalt (Co)	0.08±0^{e}	0.09±0.00^{d}	0.07±0^{f}	0.99±0^{a}	0.18±0.00^{c}	0.21±0^{b}
Copper (Cu)	2.98±0.27^{c}	2.30±0.02^{d}	3.025±0.38^{c}	5.4±0.29^{a}	4±0.07^{b}	5.7±0.06^{a}
Zinc (Zn)	103.24±1.77^{b}	88.41±0.54^{c}	66.01±1.04^{e}	102.27±0.18^{b}	107.09±1.64^{a}	78.37±0.55^{d}
Selenium (Se)	1.75±0.13^{e}	2.03±0.02^{cd}	2±0^{d}	2.17±0.02^{b}	2.28±0.02^{a}	2.13±0.03^{bc}
Ultra-trace minerals						
Arsenic (As)	1.37±0.04d^{e}	1.44±0.02^{d}	1.33±0.03^{e}	5.23±0.09^{a}	4.65±0.005^{b}	3.93±0.025^{c}
Cadmium (Cd)	0.2±0^{a}	0.17±0^{b}	0.13±0.01^{d}	0.16±0.005^{c}	0.12±0^{e}	0.06±0.00^{f}
Lead (Pb)	0.33±0.01^{de}	0.38±0.14^{d}	0.23±0.03^{e}	3.59±0.01^{a}	1.73±0.01^{b}	0.93±0.05^{c}
Vanadium (V)	0.74±0.02^{c}	0.59±0.005^{e}	0.45±0^{f}	2.31±0.005^{a}	0.99±0.03^{b}	0.7±0^{d}
Nickel (Ni)	0.99±0.08^{c}	1.36±0.04^{b}	0.28±0.01^{e}	2.41±0.09^{a}	0.66±0.1^{d}	1.01±0.01^{c}

Values are mean ± SD, Na/K = Sodium to potassium ratio, values within same rows with different superscripts are statistically different at $P < 0.05$.

iv. Amino acid profile of RUFPs

The amino acid profiles of the RUFPs developed from five fish species are presented in Table 12 and 13, expressed as the mean ± SD in µg/mg. The amounts of threonine, valine, methionine, isoleucine, leucine, phenylalanine, tryptophan, and lysine were highest in the RUFPs developed from the whole Punti. The level of arginine was highest in the Punti muscle powder (9.39 ± 0.04 µg/mg) and lowest in the Chapila muscle powder (0.38 ± 0.03 µg/mg). The amount of glycine was highest in the whole Punti powder (1.96 ± 0.02 µg/mg)

and lowest in the gutted Anchovy powder (0.18±0.02 µg/mg). Among all the RUFPs, the level of non-essential amino acids (NEAA) was highest in the Sardine muscle Punti powder (10.23±0.02 µg/mg) and lowest in the Chapila muscle powder (0.14 ± 0.05 µg/mg). Among all RUFPs, the number of essential amino acids (EAA) was higher than the quantity of NEAA. The EAA-to-NAA ratio is a metric used to assess protein quality; it indicated that all the RUFPs had high-quality protein. According to the present study, the developed RUFPs could be used to form food supplement products rich in amino acids. EAA plays a vital role in human protein synthesis. Because the human body cannot synthesize EAA, it is necessary to supplement them in the diet. In this sense, the height and weight of children who were fed a diet fortified with RUFPs increased significantly (Sen, 2005). In addition, glutamate has the potential to act as a fuel for metabolism in the small intestine and regulate nerve function. Therefore, RUFP-fortified foods could increase the nutritional value of the diet and be beneficial to human health.

Table 12. Amino acid (µg/mg±SD) profile of RUFPs developed from Chapila, Punti and Kachki fish in Bangladesh

Amino Acid	Chapila			Punti			Kachki
	Whole	Gutted	Muscle	Whole	Gutted	Muscle	Whole
Essential Amino Acid (EAA)							
Histidine	nd	nd	nd	2.02 ± 0.02^{c}	2.17 ± 0.07^{b}	2.93 ± 0.03^{a}	0.55 ± 0.02^{d}
Arginine	0.67 ± 0.02^{f}	0.79 ± 0.02^{e}	0.38 ± 0.03^{g}	5.82 ± 0.02^{c}	7.17 ± 0.02^{b}	9.39 ± 0.04^{a}	3.85 ± 0.02^{d}
Threonine	3.04 ± 0.03^{d}	1.81 ± 0.03^{f}	0.70 ± 0.02^{g}	5.26 ± 0.03^{a}	5.14 ± 0.01^{b}	3.97 ± 0.02^{c}	2.61 ± 0.02^{e}
Valine	1.37 ± 0.02^{e}	0.40 ± 0.02^{f}	0.27 ± 0.02^{g}	4.63 ± 0.02^{a}	3.88 ± 0.02^{b}	3.43 ± 0.02^{c}	1.74 ± 0.006^{d}
Methionine	0.34 ± 0.02^{e}	0.11 ± 0.01^{f}	0.13 ± 0.02^{f}	2.08 ± 0.01^{a}	1.86 ± 0.03^{b}	1.76 ± 0.03^{c}	0.81 ± 0.03^{d}
Isoleucine	0.86 ± 0.02^{e}	0.23 ± 0.02^{f}	0.19 ± 0.02^{g}	4.67 ± 0.02^{a}	3.73 ± 0.03^{b}	3.33 ± 0.02^{c}	1.04 ± 0.02^{d}
Leucine	1.23 ± 0.02^{e}	0.35 ± 0.01^{f}	0.31 ± 0.02^{g}	7.35 ± 0.03^{a}	6.16 ± 0.02^{b}	5.73 ± 0.02^{c}	1.68 ± 0.03^{d}
Phenylalanine	0.61 ± 0.02^{e}	0.24 ± 0.01^{f}	0.24 ± 0.01^{f}	2.89 ± 0.02^{a}	2.55 ± 0.03^{b}	2.49 ± 0.03^{c}	0.97 ± 0.01^{d}
Tryptophan	0.99 ± 0.01^{e}	0.33 ± 0.01^{g}	0.48 ± 0.02^{f}	3.86 ± 0.02^{a}	3.36 ± 0.02^{b}	1.70 ± 0.02^{c}	1.52 ± 0.03^{d}
Lysine	1.64 ± 0.01^{d}	0.81 ± 0.02^{e}	1.26 ± 0.005^{d}	7.37 ± 0.02^{b}	8.56 ± 0.04^{a}	8.40 ± 0.03^{a}	2.93 ± 0.59^{c}
∑EAA	1.19 ± 0.8^{b}	0.56 ± 0.53^{b}	0.44 ± 0.35^{b}	4.60 ± 1.93^{a}	4.46 ± 2.24^{a}	4.31 ± 2.69^{a}	1.77 ± 1.06^{ab}
Non-essential Amino Acid (NAA)							
Alanine	1.36 ± 0.02^{e}	0.34 ± 0.01^{f}	0.19 ± 0.02^{g}	6.15 ± 0.02^{a}	5.49 ± 0.01^{b}	4.91 ± 0.04^{c}	2.26 ± 0.02^{d}
Proline	0.53 ± 0.02^{e}	0.11 ± 0.02^{f}	0.12 ± 0.005^{f}	2.24 ± 0.02^{a}	1.71 ± 0.02^{b}	1.44 ± 0.03^{c}	1.11 ± 0.02^{d}
Tyrosine	0.12 ± 0.03^{e}	nd	0.17 ± 0.01^{e}	1.56 ± 0.03^{c}	1.75 ± 0.03^{b}	2.58 ± 0.03^{a}	0.93 ± 0.02^{d}
Aspartic acid	0.09 ± 0.01^{e}	0.08 ± 0.01^{e}	0.06 ± 0.005^{f}	0.43 ± 0.02^{b}	0.26 ± 0.02^{c}	0.54 ± 0.01^{a}	0.18 ± 0.02^{d}
Glutamic acid	0.97 ± 0.02^{e}	0.25 ± 0.004^{f}	0.17 ± 0.02^{g}	3.95 ± 0.02^{a}	3.12 ± 0.03^{c}	3.31 ± 0.02^{b}	1.19 ± 0.02^{d}
Asparagine	nd	nd	nd	nd	nd	nd	0.17±0.01

Serine	nd	nd	nd	0.65±0.02^{c}	0.89±0.02^{b}	1.32±0.03^{a}	0.50±0.02^{d}
Glutamine	0.08±0.01^{e}	nd	nd	1.01±0.03^{a}	0.84±0.02^{b}	0.63±0.02^{c}	0.32±0.03^{d}
Glycine	0.33±0.02^{d}	nd	nd	1.96±0.02^{a}	1.70±0.10^{b}	1.66±0.03^{b}	0.46±0.02^{c}
∑NAA	0.50±0.49^{a}	0.20±0.12^{a}	0.14±0.05^{a}	2.24±1.93^{a}	1.97±1.66^{a}	2.05±1.48^{a}	0.79±0.67^{a}
EAA/ NAA	2.38	2.8	3.14	2.05	2.26	2.10	2.24
∑AA	14.23±0.15^{e}	5.72±0.22^{f}	4.66±0.01^{g}	63.89±0.10^{a}	60.35±0.12^{b}	59.52±0.12^{c}	24.83±0.61^{d}

Values are mean ± SD, nd = Not detected, AA = Amino acids, values within same rows with different superscripts are statistically different at $P < 0.05$.

Table 13. Amino acid composition (mg/g±SD) of Ready-to-use fish products (RUFPs; Fish powder) developed from sundried Anchovy and Sardine fish in Bangladesh

Amino acids	**Anchovy**			**Sardine**		
	Whole	**Gutted**	**Muscle**	**Whole**	**Gutted**	**Muscle**
Essential Amino Acid (EAA)						
Arginine	2.44±0.03^{e}	4.08±0.04^{c}	4.70±0.02^{b}	3.22±0.02^{d}	4.73±0.04^{b}	5.22±0.03^{a}
Histidine	nd	nd	nd	0.38±0.01^{c}	0.78±0.01^{b}	0.83±0.02^{a}
Isoleucine	0.54±0.01^{e}	0.63±0.02^{d}	0.58±0.03^{e}	1.65±0.03^{a}	1.19±0.02^{c}	1.45±0.03^{b}
Leucine	0.95±0.03^{d}	1.13±0.02^{c}	0.94±0.03^{d}	2.79±0.03^{a}	2.28±0.04^{b}	2.82±0.03^{a}
Lysine	2.55±0.04^{e}	2.87±0.02^{c}	2.70±0.03^{d}	1.98±0.02^{f}	3.72±0.03^{a}	3.53±0.01^{b}
Methionine	0.64±0.02^{e}	0.79±0.03^{d}	0.68±0.03^{e}	0.99±0.03^{b}	0.84±0.01^{c}	1.24±0.02^{a}
Threonine	3.34±0.03^{c}	3.04±0.03^{d}	2.13±0.03^{f}	4.41±0.02^{a}	4.25±0.02^{b}	2.94±0.03^{e}
Valine	0.92±0.02^{f}	1.12±0.03^{d}	1±0.03^{e}	2.14±0.03^{a}	1.89±0.00^{c}	2.10±0.01^{b}
Phenylalanine	0.5±0.02^{e}	0.6±0.02^{d}	0.53±0.03^{e}	1.02±0.01^{b}	0.79±0.03^{c}	1.07±0.02^{a}
Tryptophan	0.66±0.03^{f}	1.22±0.03^{e}	1.42±0.03^{d}	1.80±0.02^{b}	1.47±0.02^{c}	1.96±0.01^{a}
∑EAA	12.54±0.02	15.48±0.03	14.68±0.03	20.38±0.02	21.94±0.02	23.16±0.01
Non-essential Amino Acid (NAA)						
Alanine	0.89±0.02^{f}	0.97±0.03^{e}	1.08±0.03^{d}	3.73±0.02^{c}	3.78±0.02^{b}	4.44±0.03^{a}
Aspartic acid	0.08±0.02^{cd}	0.07±0.00^{d}	0.11±0.02^{bc}	0.12±0.03^{b}	0.07±0.01^{d}	0.15±0.00^{a}
Glutamic acid	0.47±0.03^{f}	0.84±0.02^{e}	1.07±0.02^{d}	1.91±0.04^{b}	1.62±0.03^{c}	2.83±0.02^{a}

Serine	0.17±0.02^{c}	0.07±0.00^{e}	0.09±0.00^{d}	0.19±0.02^{c}	0.31±0.00^{a}	0.22±0.00^{b}
Glutamine	0.29±0.02^{b}	0.30±0.02^{b}	0.37±0.01^{a}	nd	nd	nd
Glycine	0.32±0.00^{d}	0.18±0.02^{f}	0.22±0.03^{e}	0.87±0.02^{a}	0.53±0.02^{c}	0.59±0.03^{b}
Proline	0.34±0.01^{d}	0.37±0.01^{d}	0.43±0.00^{c}	1.12±0.02^{a}	0.96±0.02^{b}	1.13±0.02^{a}
Tyrosine	0.34±0.02^{c}	0.35±0.01^{c}	0.17±0.01^{d}	0.48±0.02^{b}	0.86±0.03^{a}	0.87±0.02^{a}
∑NAA	2.9±0.02	3.15±0.01	3.54±0.01	8.42±0.02	8.13±0.01	10.23±0.02
∑AA	15.44±0.21^{f}	18.63±0.11^{d}	18.22±0.33^{e}	28.8±0.22^{c}	30.07±0.21^{b}	33.39±0.02^{a}

Values are mean ± SD, nd = Not detected, AA = Amino acids, values within same rows with different superscripts are statistically different at $p < 0.05$.

v. Fatty acid profile of RUFPs

The fatty acid composition of all the RUFPs is presented in Table 14 and 15, expressed as the mean ± SD in mg/100 g. Saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), PUFA, n-6 PUFA, n-3 PUFA, fatty acid and dimethyl acetal (DMA), the PUFA/SFA ratio, EPA + DHA, and the n-6/n-3 ratio were all used to calculate the total fatty acids. SFA was highest in the whole Sardine powder (4957.29±18.56a mg/100 g) and lowest in the Anchovy muscle powder (1192.29±2.41 mg/100 g). Among all the Fish powders, the highest amount of PUFA was found in the whole Sardine powder (2619.79±0.86 mg/100 g) and the lowest was found in the Anchovy muscle powder (841.73±4.19 mg/100 g). The EPA + DHA value was the highest in the whole Sardine powder (1303.69 mg/100 g) and lowest in the Punti muscle powder (237.14 mg/100 g). In the developed RUFPs, the n-6/n-3 ratio was 0.25–1.73, which is lower than 4, indicating that the RUFPs are safe for consumers. The total amount of fatty acid was highest in the whole Punti powder (10879.24 ± 32.04 mg/100 g) and lowest in the Anchovy muscle powder (2567.47±10.22 mg/100 g).

Table 14. Fatty acid composition (mg/100g±SD) of total lipid obtained from RUFPs of Chapila, Punti and Kachki fish in Bangladesh

Fatty Acids	Chapila			Punti			Kachki
	Whole	Gutted	Muscle	Whole	Gutted	Muscle	Whole
Saturated Fatty Acid (SFA)							
14:00	583.6±10.7^{b}	352.90±5.60^{c}	625.21±3.76^{a}	262.145±0.63^{d}	215.39±6.61^{e}	154.07±1.77^{f}	255.89±0.05^{d}
iso 15:0	77.82±1.29^{c}	55.91±0.53^{d}	39.21±0.17^{f}	107.635±2.60^{a}	83.44±1.28^{b}	55.65±1.32^{d}	44.11±0.74^{e}
anteiso 15:0	15.32±1^{c}	10.56±0.15d	9.05±0.18^{e}	31.20±1.07^{a}	24.22±0.31^{b}	16.15±0.81^{c}	10.03±0.08^{de}
15:00	163.81±0.59^{a}	86.29±1.40^{e}	157.75±0.44^{b}	140.33±2.85^{c}	108.75±1.70^{d}	75.80±1.86^{f}	68.19±0.98^{g}
iso 16:0	19.41±0.08^{d}	14.49±0.47^{e}	12.66±0.54^{f}	59.56±0.52^{a}	44.77±0.22^{b}	29.12±1.13^{c}	12.97±0.54^{f}

Fatty Acids	Chapila			Punti			Kachki
	Whole	Gutted	Muscle	Whole	Gutted	Muscle	Whole
16:00	2317.34±8.26^{b}	1716.93±16.47^{e}	1813.66±5.94^{d}	2593.94±6.51^{a}	2056.46±23.76^{c}	1401.88±16.14^{g}	1686.90±10.63^{f}
iso 17:0	54.91±0.56^{d}	41.04±0.04^{e}	31.85±0.15^{f}	212.09±1.19^{a}	163.95±3.32^{b}	108.71±0.34^{c}	32.74±1.31^{f}
anteiso 17:0	27.77±1.36^{c}	21.31±0.49^{cd}	14.94±0.5^{d}	79.16±3.29^{a}	44.13±13.92^{b}	39.43±0.29^{b}	16.51±0.55^{d}
18:00	576.63±0.68^{c}	437.11±5.07^{e}	409.41±2.43^{f}	1016.24±2.72^{a}	789.59±6.64^{b}	544.37±7.84^{d}	583.64±5.91^{c}
19:00	22.82±1.09^{c}	16.78±0.33^{e}	17.29±0.18^{e}	35.54±0.39^{a}	26.07±1.22^{b}	19.45±1.28^{d}	17.57±1.37^{e}
20:00	27.29±0.49^{c}	20.19±1.05^{e}	18.98±0.47^{e}	45.65±0.77^{a}	34.91±1.11^{b}	24.65±0.55^{d}	25.68±0.89^{d}
22:00	20.32±1.09^{c}	15.47±0.45^{de}	14.35±0.05^{e}	29.17±0.13^{a}	21.27±1.13^{c}	16.53±1.19^{d}	25.47±0.04^{b}
24:00:00	26.18±0.64^{b}	25.50±0.12^{b}	18.45±0.15^{c}	14.25±0.27^{d}	11.95±0.78^{e}	8.44±0.66^{f}	55.76±0.11^{a}
ΣSFA	3933.19±18.72^{b}	2814.47±29.25^{e}	3182.78±11.61^{d}	4626.89±2.38^{a}	3624.87±27.44^{c}	2494.22±32.58^{f}	2835.44±20.02^{e}
Mono Unsaturated Fatty Acid (MUFA)							
16:1n-9	67.30±0.56^{c}	48.47±0.45^{e}	35.74±0.54^{g}	104.995±1.45^{a}	81.81±1.6^{b}	55.98±0.75^{d}	42.045±0.45^{f}
16:1n-7	626.50±4.82^{a}	472.68±4.12^{b}	286.15±0.57^{f}	421.94 ± 2.04^{c}	362.25±6.53^{d}	254.985±1.58^{g}	294.84±2.26^{e}
17:01	47.51±0.03^{c}	28.87±1.04^{f}	44.26±0.37^{d}	78.48±1.19^{a}	60.71±1.04^{b}	39.47±0.39^{e}	26.22±0.21^{g}
18:1n-9	689.51±2.94^{d}	493.93±4.13^{f}	532.86±1.49^{e}	2731.97±13.29^{a}	2158.57±13.38^{b}	1430.46±4.68^{c}	499.48±5.23^{f}
18:1n-7	256.95±0.22^{c}	196.77±3.36^{d}	148.08±0.36^{f}	360.30±3.69^{a}	289.11±3.38^{b}	199.93±2.35^{d}	160.86±0.29^{e}
20:1n-11	21.43±0.59^{d}	17.04±1.2^{e}	11.09±0.40^{g}	136.13±2.03^{a}	103.94±1.05^{b}	65.93±1.72^{c}	13.82±0.96^{f}
20:1n-9	52.70±0.79^{c}	43.73±0.55^{d}	30.20±0.52^{e}	71.97±1.87^{a}	56.55±2.79^{b}	42.49±0.13^{d}	30.83±3.78^{e}
20:1n-7	9.28±0.25^{a}	6.28±0.53^{b}	4.59±0.93^{b}	10.51±0.75^{a}	9.43±1.66^{a}	6.06±1.27^{b}	6.31±1.58^{b}
22:1n-11	7.40±2.02^{d}	11.70±1.47^{bcd}	7.84±3.29^{d}	25.57±8.20^{a}	15.87±0.005^{bc}	17.46±0.19^{b}	9.96±1.41^{cd}
22:1n-9	12.15±0.81^{a}	8.57±0.44^{bc}	5.37±0.48^{d}	9.84±2.25^{b}	10.21±0.01^{b}	4.79± 0.005^{d}	7.55±0.01^{c}
24:1n-9	12.83±0.92^{b}	12.76±0.69^{b}	8.11±0.71^{c}	nd	6.76±1.62^{c}	nd	18.96±0.24^{a}
ΣMUFA	1803.54±0.32^{d}	1340.78±17.96^{e}	1114.26±7.06^{f}	3951.69±13.39^{a}	3142.15±5.76^{b}	2115.14±10.25^{c}	1110.84±15.98^{f}
Poly Unsaturated Fatty acid (PUFA)							
18:2n-6	313.20±0.91^{d}	251.69±3.32^{e}	166.945±1.08^{g}	853.86±0.74^{a}	729.21±4.51^{b}	476.56±3.49^{c}	188.70±0.89^{f}
18:3n-6	52.94±1.23^{a}	44.97±0.46^{b}	18.34±0.80^{e}	45.31±0.25^{b}	37.20 ±0.79^{c}	24.88±0.86^{d}	12.35±6.06^{f}
20:2n-6	26.95±0.32^{d}	24.44±1.17^{d}	18.805±0.93^{e}	87.43±5.81^{a}	70.03±0.18^{b}	46.48±3.0^{c}	15.69±0.34^{e}
20:3n-6	33.92±0.31^{c}	28.82±0.36^{d}	19.33±0.61^{f}	56.57±1.79^{a}	49.30±0.04^{b}	35.09±1.04^{e}	23.595±0.99^{e}
20:4n-6	184.99±2.73^{d}	159.12±1.69^{e}	152.32±0.62^{f}	294.3±0.34^{a}	266.245±0.41^{b}	203.47±1.63^{c}	185.45±2.36^{d}
22:4n-6	17.54±0.34^{d}	12.89±0.44^{f}	15.96±0.67^{e}	45.24±0.50^{a}	37.58±1.5^{b}	28.77±0.18^{c}	12.61±1.17^{f}
22:5n-6	98.67±1.20^{b}	89.76±0.59^{c}	79.40±0.85^{d}	56.10±3.76^{e}	48.49±0.99^{f}	40.15±0.40^{g}	123.23±0.45^{a}
Σn-6 PUFA	728.19±2.13^{d}	611.67±3.75^{e}	471.09±0.91^{g}	1438.8±12.68^{a}	1238.05±7.97^{b}	855.38±10.59^{c}	561.61±6.55^{f}
18:3n-3	389.09±4.14^{a}	293.30±1.12^{c}	179.43±0.63^{e}	365.11±1.87^{b}	297.26±11.42^{c}	204.42±1.75^{d}	204.68±0.61^{d}
18:4n-3	124.80±1.86^{a}	87.3±0.89^{b}	86.91±0.45^{b}	43.405±2.06^{d}	37.74±0.35e	26.31±0.04^{f}	50.45±0.99^{c}
20:3n-3	19.27±0.05^{a}	15.29±0.63^{bc}	11.82±0.23^{d}	19.47±0.35^{a}	15.76±0.22^{b}	11.87±0.12^{d}	14.67±0.83^{c}
20:4n-3	52.02±1.61^{a}	41.67±0.18^{b}	31.28±0.99^{c}	21.57±1.23^{d}	17.18±0.54^{e}	13.23±0.51^{f}	43.15±1.26^{b}
20:5n-3 (EPA)	190.66±1.79^{b}	154.47±0.10^{d}	161.78±0.88^{c}	120.94±3.66^{e}	122.97±1.69^{e}	99.15±2.18^{f}	200.25±2.47^{a}
21:5n-3	11.09±1.98^{abc}	9.41±0.54^{c}	7.12±0.22^{d}	11.67±1.49^{ab}	12.16±0.23^{a}	7.11±0.31^{d}	9.99±1.25^{bc}

Fatty Acids	Chapila			Punti			Kachki
	Whole	Gutted	Muscle	Whole	Gutted	Muscle	Whole
22:5n-3	54.91±0.22^{c}	45.46±0.04^{e}	49.36±2.64^{d}	70.2±2.27^{a}	60.04±1.37^{b}	48.98±2.79^{d}	55.65±0.98^{c}
22:6n-3 (DHA)	308.49±4.95^{c}	263.94±0.07^{d}	336.43±3.42^{b}	181.30±0.93^{e}	183.56±4.15^{e}	137.99±0.24^{f}	484.02±0.37^{a}
Σn-3 PUFA	1150.33±0.59^{a}	910.83±0.99^{c}	864.12±4.01^{d}	833.66±9.005^{e}	746.66±3.59^{f}	549.04±2.41^{g}	1062.85±6.26^{b}
16:02	33.09±0.73^{a}	26.73±0.02^{b}	11.47±0.19^{e}	20.48±0.94^{c}	19.53±1.05^{c}	14.52±0.38^{d}	13.43±0.22^{d}
16:03	20.275±0.88^{a}	17.36±0.89^{b}	4.59±0.09^{e}	7.735±0.28^{d}	13.49±4.14^{c}	11.79±0.27^{c}	6.27±0.66d^{e}
16:04	32.13±5.70^{a}	11.83±0.12^{c}	5.83±0.02^{d}	nd	25.04±0.79^{b}	14.27±1.55^{c}	10.34±0.18^{c}
Σ16:02-04	85.50±4.09^{a}	55.92±1.03^{b}	21.89±0.26^{e}	28.215±0.66^{d}	58.05±5.99^{b}	40.58±2.19^{c}	30.04±0.62^{d}
ΣPUFA	1964.03±1.38^{c}	1578.41±3.78^{e}	1357.09±4.67^{g}	2300.67±21.02^{a}	2042.75±17.55^{b}	1444.99±15.18^{f}	1654.49±12.20^{d}
Fatty acids and Dimethyl Acetal (DMA)							
16:0 DMA	16.48±3.02^{b}	12.47±0.62^{b}	11.39±0.97^{b}	17.835±0.755^{b}	29.89±15.11^{a}	12.33±0.13^{b}	13.25±0.89^{b}
18:0 DMA	23.64±1.94^{ab}	25.25±0.07^{ab}	11.01±0.30^{d}	23.265±1.005^{b}	22.41±0.85^{b}	14.24±1.26^{c}	26.31±3.22^{a}
ΣDMA	40.11±1.09^{b}	37.71±0.69^{b}	22.41±1.28^{c}	41.1±0.25^{b}	52.30±15.96^{a}	26.56±1.13^{c}	39.56±2.33^{b}
PUFA/SFA	0.50	0.56	0.43	.50	0.56	0.58	0.58
EPA+DHA	499.15	418.41	498.21	302.24	306.53	237.14	684.27
n-6/n-3	0.63	0.67	0.55	1.73	1.66	1.56	0.53
ΣFA	7700.75±17.68^{c}	5733.66±50.99^{e}	5654.13±23.33^{f}	10879.24±32.04^{a}	8809.76±50.76^{b}	6054.35±58.01^{d}	5600.77±48.19^{f}

Values are mean ± SD, nd = Not detected, SFA = Saturated fatty acids, MUFA = Monounsaturated fatty acids, PUFA = Polyunsaturated fatty acids, DMA = Fatty acids and Dimethyl acetal; PUFA/SFA = PUFA to SFA ratio, EPA = Eicosapentaenoic acid; DHA = Docosahexaenoic acid; n-3/n-6 = n-3 to n-6 ratio, FA = Fatty acids; Values within same rows with different superscripts are statistically different at $P < 0.05$.

Table 15. Fatty acid (mg/100g±SD) of Ready-to-use fish products (RUFPs; Fish powder) developed from sundried Anchovy and Sardine fish in Bangladesh

Fatty acids	Anchovy			Sardine		
	Whole	Gutted	Muscle	Whole	Gutted	Muscle
14:00	150.32±4.18^{d}	108.75±1.09^{e}	104.68±1.60^{e}	1058.64±2.49^{a}	878.52±3.53^{b}	661.55±0.11^{c}
iso 15:0	10.32±0.24^{c}	6.03±0.17^{d}	6.32±0.23^{d}	19.37±1.7^{a}	12.91±0.92^{b}	11.60±0.49^{bc}
anteiso 15:0	5.08±0.005^{c}	3.73±00.11^{d}	3.72±0.37^{d}	10.16±0.00^{a}	6.96±0.00^{b}	4.95±0.45^{c}
15:00	34.10±0.01^{d}	25.37±0.04^{e}	25.11±0.50^{e}	90.09±0.27^{a}	70.80±0.51^{b}	59.28±0.34^{c}

Fatty acids	Anchovy			Sardine		
	Whole	Gutted	Muscle	Whole	Gutted	Muscle
iso 16:0	5.16±0.24^{b}	2.91±0.1^{c}	3.25±0.00^{c}	8.34±0.7^{a}	5.07±0.00^{b}	5.82±1.14^{b}
16:00	904.12±19.83^{d}	727.53±2.55^{e}	715.97±0.64^{e}	2969.02±27.11^{a}	2483.06±18.21^{b}	2154.44±12.34^{c}
iso 17:0	13.13±0.48^{c}	8.09±0.34^{d}	8.89±0.13^{d}	32.22±0.77^{a}	24.83±0.95^{b}	23.52±1.54^{b}
anteiso 17:0	7.52±0.58^{b}	5.18±0.54^{c}	5.33±0.23^{c}	9.47±0.95^{a}	9.07±0.65^{a}	7.93±0.31^{b}
18:00	340.51±5.20^{d}	267.38±0.14^{e}	256.77±0.45^{f}	657.92±1.61^{a}	546.17±4.62^{b}	482.99±2.32^{c}
19:00	10.25±0.09^{d}	8.03±0.37^{e}	8.61±0.215^{e}	21.44±0.26^{a}	18.25±0.26^{b}	15.50±1.18^{c}
20:00	20±0.4^{c}	14.74±0.04^{d}	14.42±0.04^{d}	31.85±1.37^{a}	28.09±1.38^{b}	21.09±0.62^{c}
22:00	23±0.67^{a}	17.29±0.96^{c}	15.44±0.33^{d}	23.63±0.05^{a}	20.88±2.07^{b}	16.65±0.02^{cd}
24:00:00	39.52±1.40^{a}	31.6±2.03^{b}	23.81±0.22^{c}	30.22±.11^{b}	30.62±3.39^{b}	17.28±0.71^{d}
ΣSFA	1563.02±33.28^{d}	1226.62±3.28^{e}	1192.29±2.41^{f}	4957.29±18.56^{a}	4129.19±15.39^{b}	3482.58±12.47^{c}
16:1n-9	5.98±0.07^{b}	4.20±0.07^{c}	4.48±0.05^{c}	6.50±0.4^{b}	18.71±1.14^{a}	4.42±0.00^{c}
16:1n-7	276.90±3.74^{d}	213.05±0.41^{e}	196.78±1.62^{f}	1574.82±11.66^{a}	1355.17±10.94^{b}	1115.44±9.76^{c}
17:01	13.48±0.63^{a}	8.99±1.81^{b}	6.75±0.02^{c}	14.86±1.44^{a}	14.46±0.09^{a}	10.68±1.68^{b}
18:1n-9	295.33±2.89^{d}	224.75±3.07^{e}	203.74±1.31^{f}	786.19±0.28^{a}	647.33±1.37^{b}	580.87±2.94^{c}
18:1n-7	109.86±1.29^{d}	87.05±0.42^{e}	83.11±0.45^{e}	429.29±4.16^{a}	360.73±4.31^{b}	309.00±0.58^{c}
20:1n-11	5.32±0.00^{c}	2.24±0.11^{e}	3.36±0.02^{d}	7.40±0.12^{a}	6.17±0.66^{b}	5.96±0.28^{b}
20:1n-9	10.33±0.15^{c}	7.37±0.39^{d}	7.14±0.09^{d}	36.11±1.61^{a}	28.82±1.475^{b}	27.97±2.26^{b}
20:1n-7	8.32±0.07^{c}	6.40±0.17^{d}	5.58±0.90^{d}	15.74±0.81^{a}	14.25±1.47^{b}	14.72±0.15^{ab}
22:1n-11	2.94±0.00^{c}	2.47±0.00^{c}	nd	13.17±2.52^{a}	11.99±0.00^{a}	9.08±0.91^{b}
22:1n-9	2.81±0.32^{e}	2.53±0.02^{e}	4.61±0.09^{d}	8.34±0.31^{a}	6.33±0.23^{b}	5.09±0.32^{c}
24:1n-9	28.45±1.36^{a}	22.83±0.51^{b}	17.91±1.19^{c}	27.58±0.14^{a}	21.98±1.41^{b}	14.82±2.27^{d}
ΣMUFA	758.24±8.10^{d}	580.63±7.02^{e}	533.46±3.62^{f}	2916.94±14.28^{a}	2479.92±9.9^{b}	2095.80±13.04^{c}
18:2n-6	44.28±0.09^{d}	34.54±0.39^{e}	35.98±0.79^{e}	170.89±2.85^{a}	129.25±2.44^{b}	123.60±2.41^{c}
18:3n-6	4.48±0.39^{c}	4.12±0.22^{c}	4.18±0.19^{c}	41.32±1.09^{a}	27.89±1.12^{b}	26.87±1.16^{b}
20:2n-6	8.56±0.34^{bc}	8.98±2.14^{bc}	7.92±0.04^{c}	10.85±3.08^{abc}	11.25±0.6^{ab}	12.57±0.94^{a}

Fatty acids	Anchovy			Sardine		
	Whole	Gutted	Muscle	Whole	Gutted	Muscle
20:3n-6	9.19±0.26^{c}	7.51±0.26^{d}	7.48±0.17^{d}	30.60±1.15^{a}	23.99±0.82^{b}	24.26±0.63^{b}
20:4n-6	79.74±0.54^{d}	80.55±1.13^{d}	76.22±0.16^{e}	154.34±1.46^{a}	121.38±0.49^{c}	126.45±2.82^{b}
22:4n-6	7.17±0.43^{c}	7.64±0.02^{c}	7.31±0.03^{c}	15.55±1.51^{a}	7.56±1.64^{c}	9.63±0.17^{b}
22:5n-6	31.37±0.43^{d}	31.77±0.65^{d}	31.76±0.37^{d}	44.89±0.77^{b}	41.37±2.61^{c}	49.15±3.52^{a}
Σn-6 PUFA	184.76±3.08^{d}	175.08±3.85^{e}	170.83±1.29^{e}	468.42±11.9^{a}	362.69±1.075^{c}	372.49±2.87^{b}
18:3n-3	24.48±0.32^{d}	19.07±0.20^{e}	17.71±0.99^{f}	94.04±0.7^{a}	61.32±0.54^{b}	57.40±0.93^{c}
18:4n-3	13.31±0.15^{d}	11.94±0.12^{de}	11.22±0.28^{e}	126.38±1.19^{a}	91.07±2.17^{b}	81.29±0.98^{c}
20:3n-3	4.12±o.39^{c}	4.02±0.19^{c}	3.06±0.04^{c}	9.66±1.25^{a}	7.41±0.45^{b}	6.74±0.42^{b}
20:4n-3	12.82±0.48^{c}	12.79±0.29^{c}	11.55±0.45^{c}	51.41±2.16^{a}	38.20±0.47^{b}	37.92±1.29^{b}
20:5n-3	158.92±4.49^{d}	169.58±1.67^{d}	160.03±0.86^{d}	816.03±7.92^{a}	611.39±13.29^{c}	626.34±0.25^{b}
21:5n-3	6.52±0.77^{c}	6.10±0.07^{c}	5.92±0.15^{c}	43.07±2.18^{a}	32.49±0.58^{b}	33.15±0.82^{b}
22:5n-3	39.83±1.59^{de}	40.97±0.26^{d}	36.53±0.35^{e}	90.34±1.02^{a}	73.82±4.98^{b}	78.40±0.45^{c}
22:6n-3	397.71±12.17^{d}	428.44±6.79^{c}	401.26±1.81^{d}	487.66±4.70^{b}	436.56±5.41^{c}	510.95±0.15^{a}
Σn-3 PUFA	657.67±19.39^{e}	692.88±8.24^{d}	647.26±2.31^{e}	1718.56±14.28^{a}	1352.25±25.81^{c}	1432.17±0.86^{b}
16:02	18.94±1.09^{d}	15.56±0.42^{e}	13.98±0.17^{e}	187.63±1.35^{a}	159.43±2.93^{b}	140.89±0.23^{c}
16:03	12.97±0.24^{d}	10.86±0.89^{de}	9.65±0.43^{e}	222.05±2.89^{a}	174.67±2.15^{b}	161.19±1.99^{c}
16:04	nd	nd	nd	23.14±7.47^{a}	25.71±0.56^{a}	8.99±0.73^{b}
Σ16:02-04	31.91±1.33^{d}	26.42±1.32^{e}	23.64±0.59^{e}	432.81±3.23^{a}	359.80±4.52^{b}	311.06±2.49^{c}
ΣPUFA	874.34±23.81^{d}	894.38±10.77^{d}	841.73±4.19^{e}	2619.79±0.86^{a}	2074.74±29.25^{c}	2115.72±4.5^{b}
16:0 DMA	17.61±0.47^{a}	15.49±0.49^{b}	14.62±0.01^{c}	9.41±0.75^{d}	6.99±0.56^{e}	7.20±0.31^{e}
18:0 DMA	33.92±0.36^{a}	28.45±0.10^{b}	21.23±0.44^{c}	17.67±0.005^{d}	nd	8.12±2.08^{e}
ΣDMA	51.52±0.11^{a}	43.95±0.39^{b}	35.86±0.43^{c}	18.24±9.58^{d}	6.99±0.56^{e}	15.32±1.77^{d}
PUFA/SFA	0.56	0.73	0.71	0.53	0.51	0.61

Fatty acids	Anchovy			Sardine		
	Whole	Gutted	Muscle	Whole	Gutted	Muscle
EPA+DHA	556.63	598.02	561.29	1303.69	1047.95	1137.29
n-6/n-3	0.28	0.25	0.26	0.27	0.27	0.26
ΣFA	3195.59±65.19^{d}	2701.63±0.49^{e}	2567.47±10.22^{f}	10494.01±31.99^{a}	8683.85±54.55^{b}	7694.10±21^{c}

Values are mean ± SD, nd = Not detected, SFA = Saturated fatty acids, MUFA = Monounsaturated fatty acids, PUFA = Polyunsaturated fatty acids, DMA = Fatty acids, and Dimethyl acetal; PUFA/SFA = PUFA to SFA ratio, EPA = Eicosapentaenoic acid; DHA = Docosahexaenoic acid; n-6/n-3. = n-6 to n-3 ratio, FA = Fatty acids; Values within the same rows with different superscripts are statistically different at $P < 0.05$.

vi. Potential contribution to RNI

The potential contribution of the RUFPs developed from whole, gutted, and muscle Chapila, Punti, Kachki, Anchovy, and Sardine fish in the RNI for different consumers – namely adults, infants, and PLW – are given in Table 16. The results show that Ca of developed Fish powders contributes 21.36–74.16%, 28.49–98.89%, and 24.27–84.28% of RNI in adults, infants, and PLW, respectively, whereas Se contributes 32.40–91.2%, 43.20–98.4% and 33.75–95% in adults, infants and PLW accordingly. Furthermore, the contribution rate of Zn was 6.65–25.73%, 8.0–30.94%, and 5.75–22.26% in adults, infants, and PLW, respectively, while 5.19–62.55%, 8.75–97.21% and 7.77–93.57% was contributed by Fe in adults, infants, and PLW correspondingly.

Table 16. The potential contribution of RUFPs in a standard portion*, recommended nutrient intake (RNI, %) for different consumers

Parameters	Ca mg/day			Se ug/day			Zn mg/day			Fe mg/day			Protein g/day			Fat g/day			LC n-3 PUFA g/day		
	A	I	PLW	A	I	PLW	A	I	PLW	A	I	PLW	A	I	PLW	A	I	PLW	A	I	PLW
Reference value**	1000	420	1100	30	9	36	11.9	6.6	17.2	17.95	7.7	15	60	20.1	74	30	30	25	1.35	0.5	1.4
Chapila																					
Whole	62.29	83.05	70.79	43.20	57.60	45.00	13.09	15.73	11.32	62.55	97.21	93.57	10.72	21.33	10.86	5.05	4.04	6.32	10.23	18.41	12.32
Gutted	50.87	67.82	57.80	52.40	69.87	54.58	14.04	16.88	12.14	24.61	38.25	36.81	13.06	25.98	13.23	3.38	2.70	4.23	8.10	14.57	9.76
Muscle	30.11	40.15	34.22	32.40	43.20	33.75	10.63	12.78	9.19	35.54	55.24	53.17	13.92	27.71	14.11	2.74	2.20	3.43	7.68	13.83	9.26
Punti																					
Whole	46.70	62.26	53.07	36.40	48.53	37.92	13.45	16.17	11.63	46.06	71.59	68.90	9.76	19.43	9.89	7.03	5.63	8.79	7.41	13.34	8.93
Gutted	45.98	61.30	52.25	38.40	51.20	40.00	18.90	22.71	16.34	12.57	19.54	18.81	11.13	22.14	11.28	6.06	4.84	7.57	6.64	11.95	8.00
Muscle	31.66	42.21	35.97	38.00	50.67	39.58	17.87	21.48	15.46	8.86	13.77	13.26	12.43	24.74	12.60	4.57	3.66	5.72	4.88	8.78	5.88
Kachki																					
Whole	43.16	57.55	49.05	59.2	78.93	61.67	25.73	30.94	22.26	14.43	22.43	21.58	11.53	22.95	11.69	5.71	4.57	7.14	9.45	17.01	11.39
Anchovy																					
Whole	74.16	98.89	84.28	70	93.33	72.92	10.41	12.51	9	7.91	12.28	11.83	11.47	22.83	11.63	4.66	3.73	5.83	5.84	10.55	7.05
Gutted	48.62	64.83	55.26	81.2	96.66	84.58	8.91	10.71	7.71	5.63	8.75	8.43	13.05	25.99	13.23	3.51	2.81	4.38	6.15	11.08	7.42
Muscle	30.39	40.52	34.54	80	98	83.33	6.65	8	5.75	5.19	8.75	7.77	14.51	28.88	14.71	2.55	2.05	3.21	5.75	10.36	6.93
Sardine																					
Whole	50.44	67.25	57.32	86.8	94	90.41	10.31	12.39	8.91	15.53	24.14	23.24	10.71	21.31	10.85	4.89	3.91	6.12	15.27	27.49	18.41
Gutted	37.28	49.71	42.36	91.2	97.33	95	10.79	12.98	9.33	14.46	22.49	21.64	12.31	24.52	12.48	4.06	3.25	5.08	12.02	21.64	14.48
Muscle	21.36	28.49	24.27	85.2	98.4	88.75	7.91	9.49	6.83	11.45	17.82	17.14	13.92	27.71	14.11	3.43	2.74	4.29	12.73	22.91	15.34

A = Adult (18-60+ years), I = Infants (7-25 months), PLW = Pregnant and lactating women (Throughout the three trimesters of pregnancy and first 12 months of lactation), *Standard portion is assumed to be 15 g/day, 12 g/day and 8 g/day for A, I and PLW, respectively. ** Lupton et al. 2002; Bogard et al. 2015

11.1.2. Household data from all study areas (intervention and control)

i.Socio-economic data from all study areas

After curing the data set (consisting of 240 individuals), a total of 196 were sorted out for final analysis. The average age of most individuals was 14.3±2.27 years. A number of adolescent age variations were found among the targeted population of adolescent girls in the areas. Among them, the Khagrachari control showed the lower average age (13.4±2.42 years).

Table 17. Mean of Household data tabulation. Here, Lc= Lakshmipur (Control), Li= Lakshmipur (intervention), Kc=Khagrachari (Control), Ki= Khagrachari (intervention), Sc=Noakhali (Control), Si= Noakhali (intervention). All data were representing average with stand

Parameters	Kc N=25	Ki N=34	Lc N=35	Li N=39	Sc N=31	Si N=32	Overall N=196
Age (years)	13.4±2.42	13.5±2.33	14.3±2.04	14.8±2.07	15.5±2.06	14.2±2.27	14.3±2.27
Education (Class 1-12)	4.48±3.83	3.21±3.28	3.31±3.95	3.03±3.22	9.06±13.4	5.31±3.88	4.62±6.55
Land (dcm.)	145±241	129±167	36.7±93.9	35.8±70.3	41.2±50.2	27.8±49.3	65.5±131

11.1.3. Biomarkers' tabulation

Vitamin B12 was found higher in 2nd phase than in 1st phase. However, average Calcium was higher in 1st Phase followed by CRP and others. Vitamin B12 was found significantly high in intervention areas which may help to focus the project to an important thing that Fish powder can increase vitamin B12 Among adolescent girls.

Table 18. Average of Biomarkers in both phases. Here, Lc= Lakshmipur (Control), Li= Lakshmipur (intervention), Kc=Khagrachari (Control), Ki= Khagrachari (intervention), Sc=Noakhali (Control), Si= Noakhali (intervention). Here, average of Vitamin B12 (pg/mL), Ferritin (ng/mL), Calcium (mmol/L), CRP (mg/L) and Iron (µmol/L) were presented with standard deviations (±SD)

phases	Kc	Ki	Lc	Li	Sc	Si	Overall
B12.1	462±142	517±186	434±187	422±204	578±137	542±119	490±176
B12.2	477±139	509±179	558±231	524±258	631±152	591±177	550±202
Cal.1	2.48±0.0891	2.48±0.107	2.43±0.102	2.48±0.113	2.48±0.0822	2.46±0.103	2.47±0.101
Cal.2	2.45±0.111	2.48±0.111	2.46±0.118	2.43±0.119	2.46±0.100	2.49±0.0901	2.46±0.110
CRP.1	0.542±0.425	0.678±0.865	0.397±0.164	0.774±1.41	1.26±2.86	0.884±1.43	0.755±1.48
CRP.2	0.568±0.493	0.749±1.10	0.500±0.617	0.434±0.277	0.633±0.495	0.839±1.14	0.615±0.763
Fe.1	12.3±4.37	13.5±4.79	10.1±4.05	11.8±5.41	11.8±4.36	16.5±4.35	12.6±4.97
Fe.2	9.90±4.09	12.1±4.80	10.3±4.65	12.1±7.97	10.5±4.68	13.9±5.44	11.5±5.65
Fer.1	49.6±36.2	50.5±21.8	32.4±18.5	33.8±23.1	41.3±29.9	42.9±22.2	41.1±25.9
Fer.2	44.4±33.9	44.5±19.2	27.0±16.4	28.6±23.4	38.3±25.0	37.0±27.7	36.0±25.0

1=1st phase, 2=2nd phase

11.1.4. Anthropometric data from study areas

Except hemoglobin, overall anthropometric parameters were higher in the second phase than the 1st phase. BMI of sampled individuals of Noakhali (intervention and control) was higher than rest of the regions.

Table 19. Average of Anthropogenic data in both phases. Here, Lc= Lakshmipur (Control), Li= Lakshmipur (intervention), Kc=Khagrachari (Control), Ki= Khagrachari (intervention), Sc=Noakhali (Control), Si= Noakhali (intervention). Here, average of Height (cm), Height(m2), Weight (kg), BMI (kg/m2), MUAC (mm), Blood Glucose (mmol/L), Hemoglobin (mg/dl), were shown with standard deviation (±SD)

Phases	Kc	Ki	Lc	Li	Sc	Si	Overall
BMI.1	19.2±3.85	18.5±2.70	18.8±2.14	19.4±2.82	19.7±2.71	19.6±3.67	19.2±2.98
BMI.2	19.2±2.90	19.8±3.26	18.4±3.70	19.9±2.44	25.9±28.6	20.3±3.78	20.6±11.9
MUAC.1	215±23.4	216±25.8	216±22.9	224±20.4	226±21.8	228±34.4	221±25.3
MUAC.2	223±32.3	225±27.9	243±26.5	253±24.5	244±25.0	242±37.5	239±30.5
Glucs.1	6.38±0.7	6.04±0.690	6.17±0.992	6.13±0.632	6.06±0.629	6.13±0.927	6.14±0.775
Glucs.2	6.39±0.9	5.72±0.801	6.05±0.811	5.99±0.628	7.66±7.74	5.96±0.758	6.26±3.18
Hmgbin.1	9.69±7.0	13.7±1.69	13.4±1.34	13.6±1.74	13.7±1.21	13.9±1.03	13.1±3.10
Hmgbin.2	13.6±1.5	13.9±1.56	13.0±1.56	11.9±1.97	13.2±1.37	12.8±1.32	13.0±1.70

1=1st phase, 2=2nd phase

On average, glucose, hemoglobin, BMI, and MUAC increment were found in intervention then control of the Noakhali region. However, average weight and height became high in control than intervention. A minimal level of increment was found in average BMI, MUAC, glucose, and hemoglobin in the case of intervention and control in the Lakshmipur region. As a result, blood pressure was found higher along the control areas for this reason. However, BMI, MUAC and Glucose showed increment in control compared to intervention samples.

11.1.5. Biomarkers

It was found that except Khagrachari intervention, vitamin B12 in all other interventions were increased in the second phase compare the first phase. The horizontal line in Fig 18 showed the ranges of vitamin B12 of a healthy person. Fig 19 shows that ferritin decreased in the second phase rather than the first phase in all samples. Fig 20 shows calcium variability between two phases among the sampling areas except for Li, calcium increased in all sampling areas. Fig 21 is indicating the iron deficiency of people in collected samples. Fig 24 shows all MUAC in normal ranges. In Fig 23, several low BMI was found accordingly. Fig 25 shows that a significant number of tube wells were polluted with high iron levels (>0.3 mg/L) over normal ranges.

Vitamin B12

With several outliers, it was found that vitamin B12 increased from the baseline to the end line in each study area. The two lines in Fig 18 is the range of a normal vitamin B12 (211-911 pg/ml) in the human body (Hospitals, M., 2022). It seems that most adolescent girls have a normal range of vitamin B12 accordingly and it increases after a diet fortifying with Fish powder.

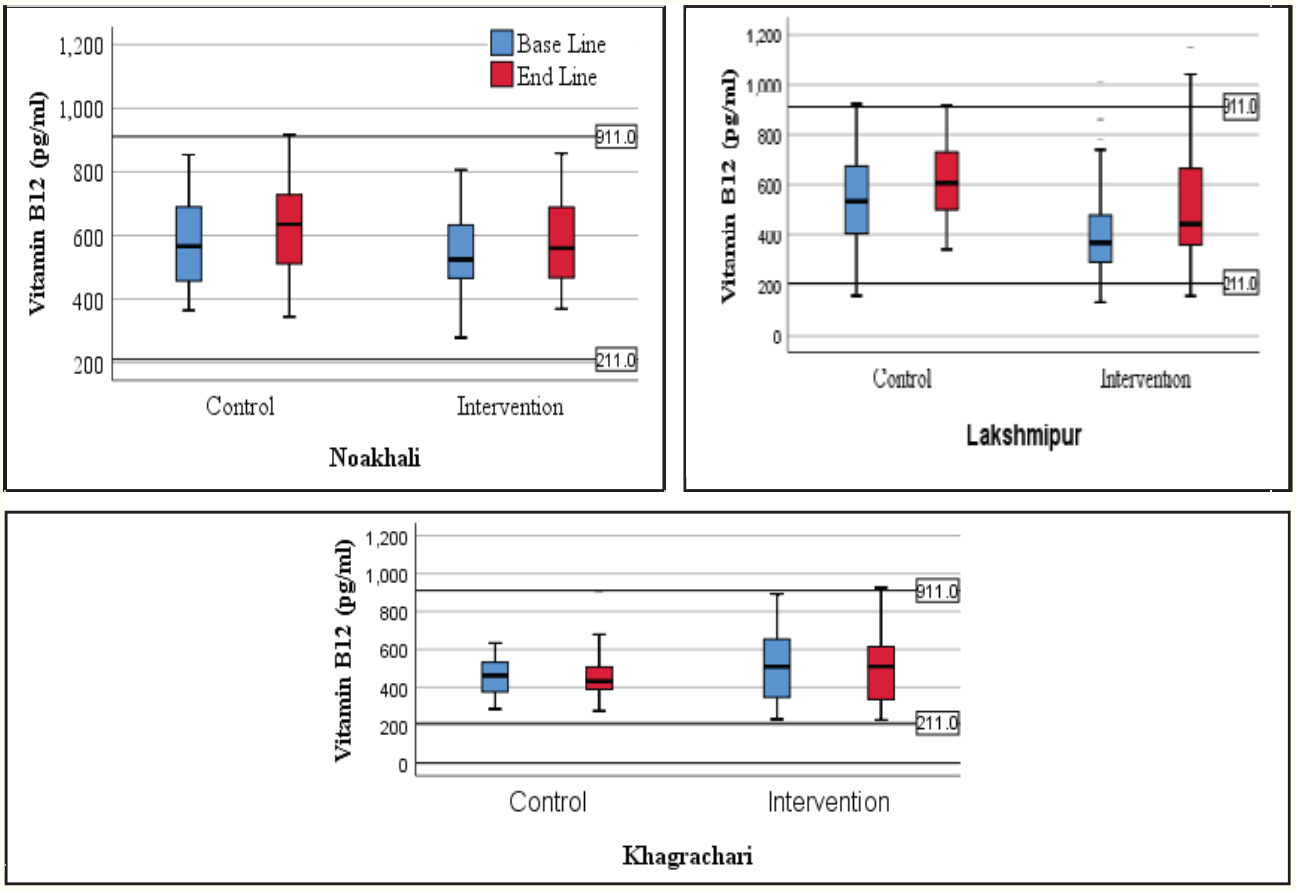


Fig 18. Vitamin B12 variability between two phases among the sampling areas with intervention

Ferritin

Except for a few cases, the ferritin levels of adolescent girls were in normal ranges (12-150 mg/dl) (Rochester, 2022). However, it decreased in the end line compared to the baseline. Notably, it decreased sharply in the control regions.

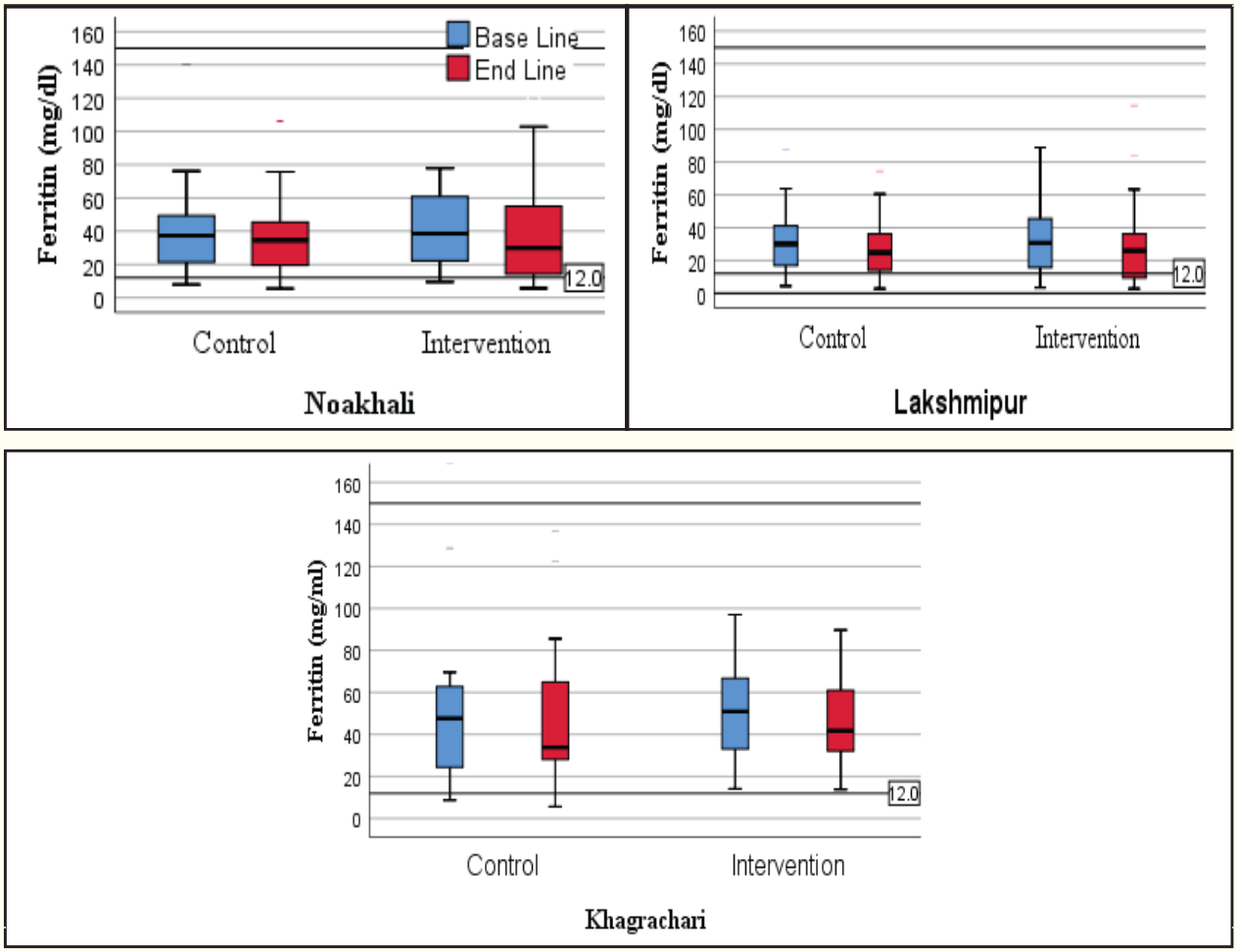


Fig 19. Ferritin variability between two phases among the sampling areas with intervention

Calcium

The calcium level of both groups at both lines was observed below the normal range (4.8-5.3 mg/dl) (Children's Hospitals, U.C.S.F.B., 2021). Besides, baseline and end line there also observed no significant differences.

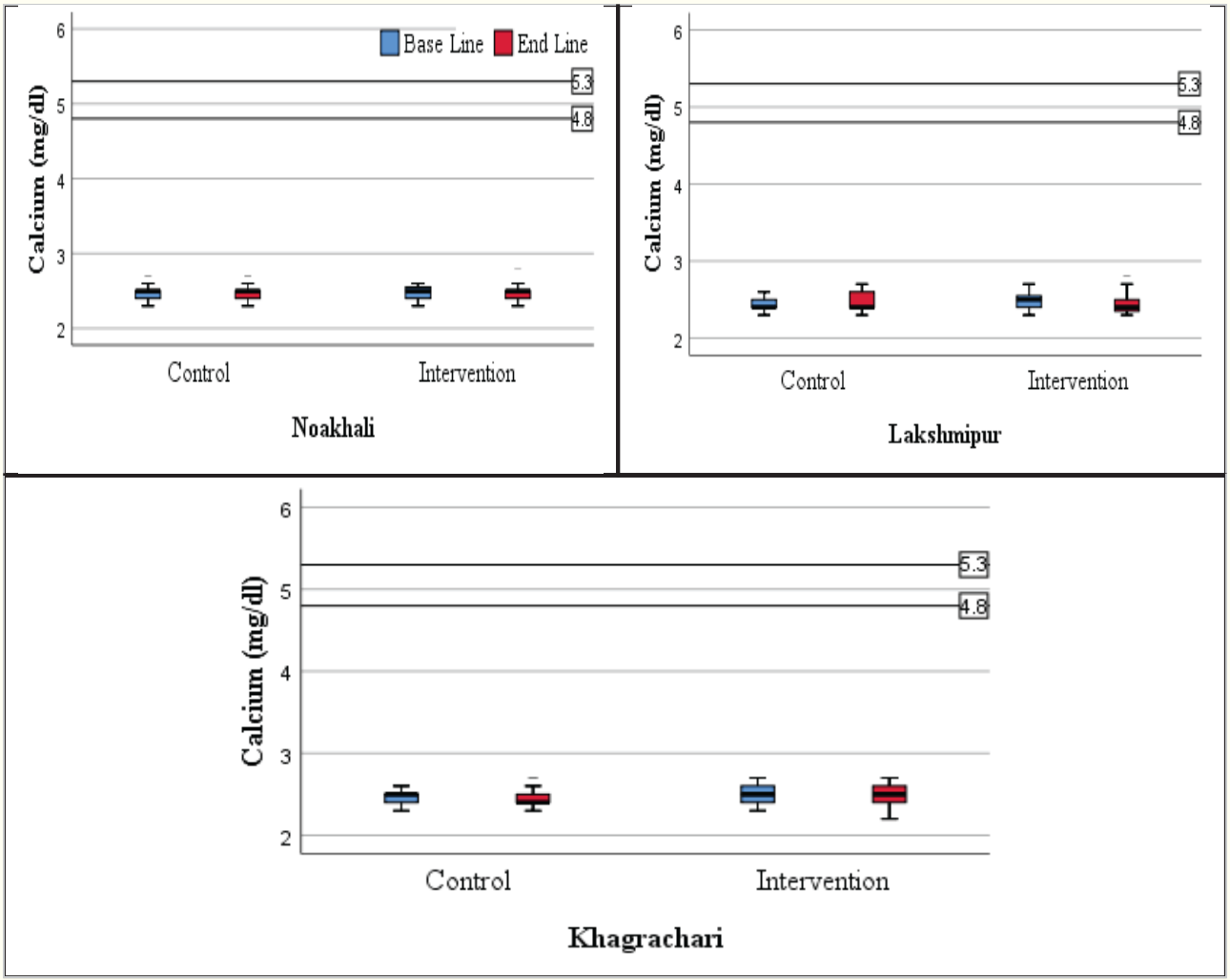


Fig 20. Calcium variability between two phases among the sampling areas with intervention

Iron (Fe)

Iron deficiency was found in most of the adolescent girls which was indicated by boxplot crossed through the low ranges of normal iron content (8.95-21.48 micromol/L) (Bishnu Prasad Devkota, M.D., 2022), especially at Lakshmipur control and intervention. Average increments of iron were found in the second phase of Lakshmipur intervention.

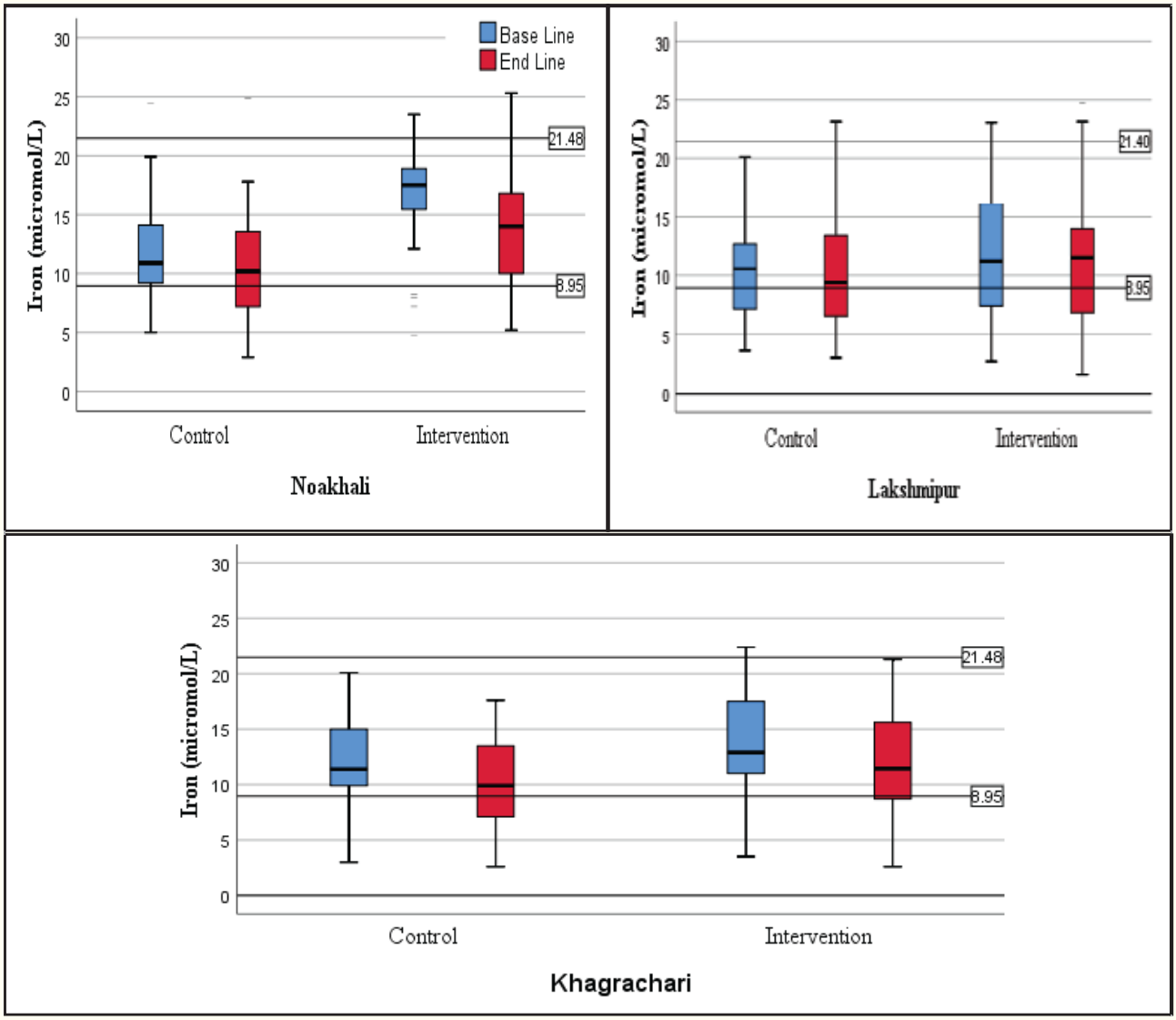


Fig 21. Iron variability between two phases among the sampling areas with intervention

Hemoglobin (Hg)

In the all research sites, the majority of teenage found to have hemoglobin levels within the normal range (lowest level and mean which were 12 and 13.5 g/dl, respectively; (Garvin Jr, J.H., 2010). While the value was lower in Noakhali and Lakshmipur, average increases in iron was reported in the second phase of the intervention group in the Khagrachari.

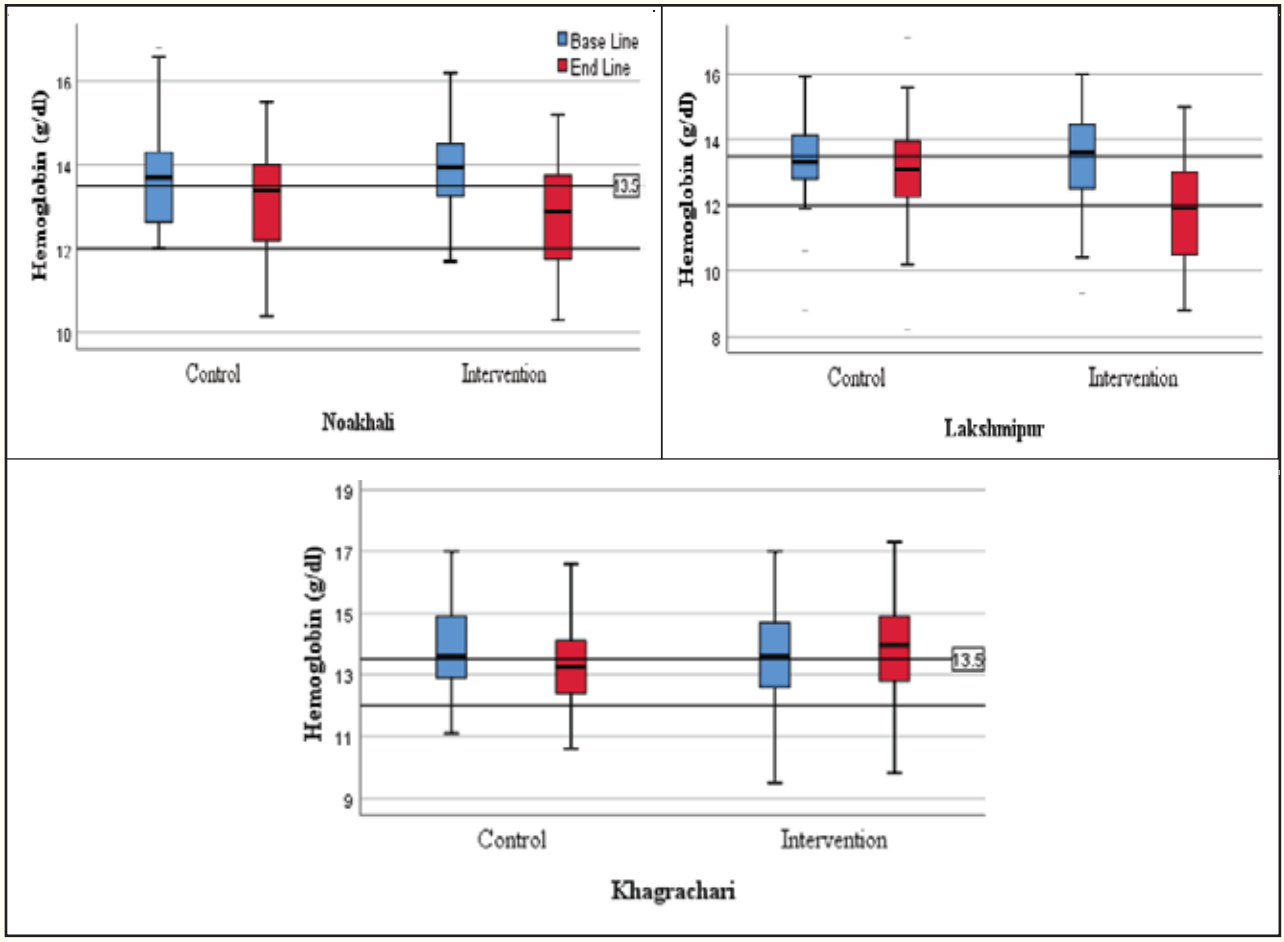
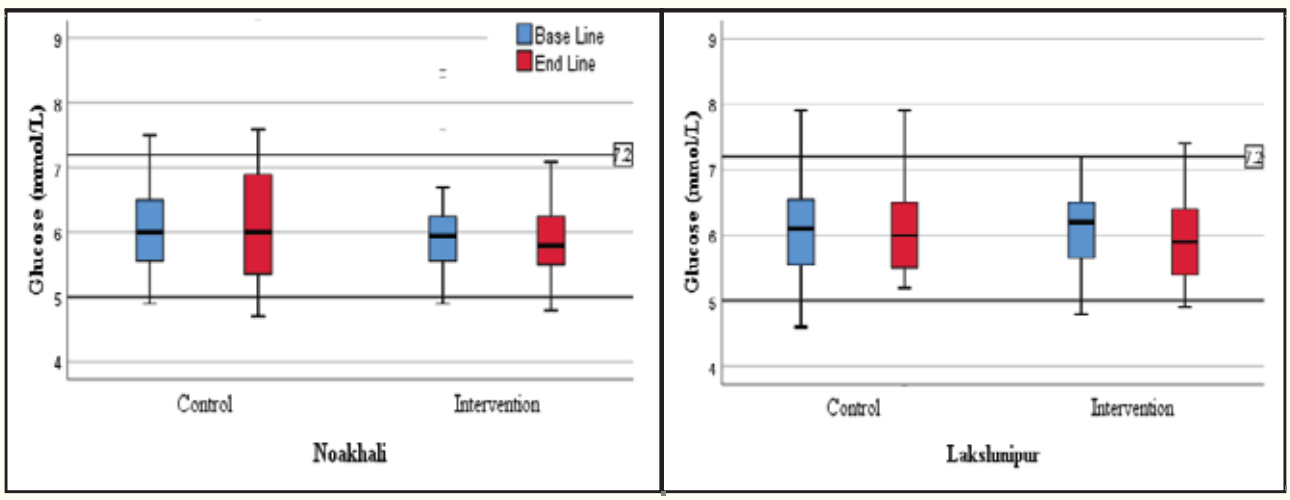


Fig 22. Hemoglobin (Hg) variability between phases among the areas with intervention and control.

Glucose

Glucose levels were found to have normal range in almost all of the adolescent girls under study (5-7.2 mmol/L; MedlinePlus, 2022). It is worth noting that among the intervention groups of the three study locations, the adolescent girls experienced a reduction in blood glucose level.



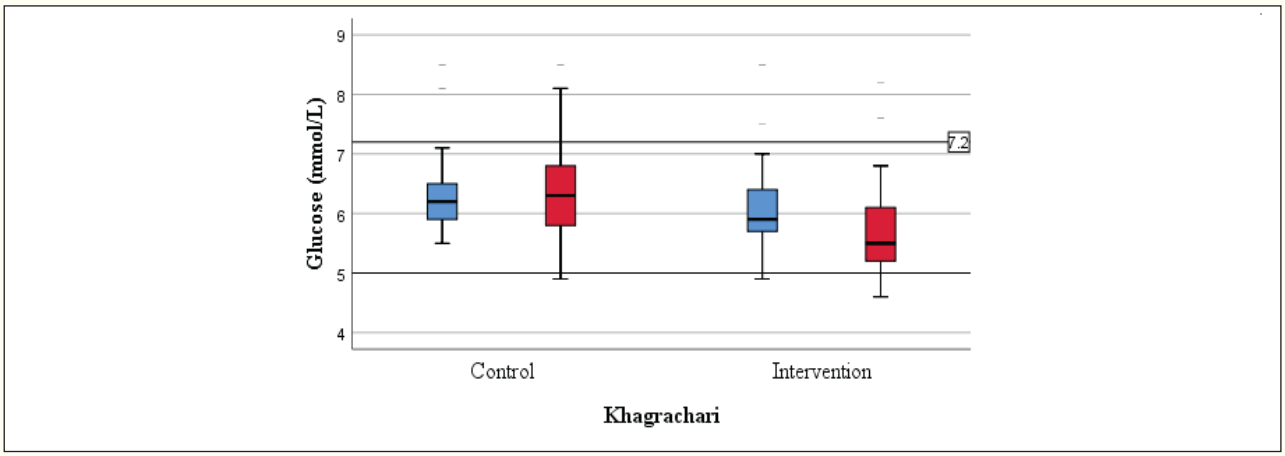


Fig 23. Glucose variability between phases among the areas with intervention and control.

MUAC (Mid-Upper Arm Circumference)

All MUAC readings were under normal ranges (185-220 mm) (Alvarez JL et al., 2018). It increased significantly in the second phase rather than the first phase. Ideal MUAC was subtracted from observed MUAC for understanding its position from the threshold of MUAC. Except for Khagrachari, the average MUAC values were higher in the rest of the study area. The highest average of MUAC was found at Lakshmipur (intervention).

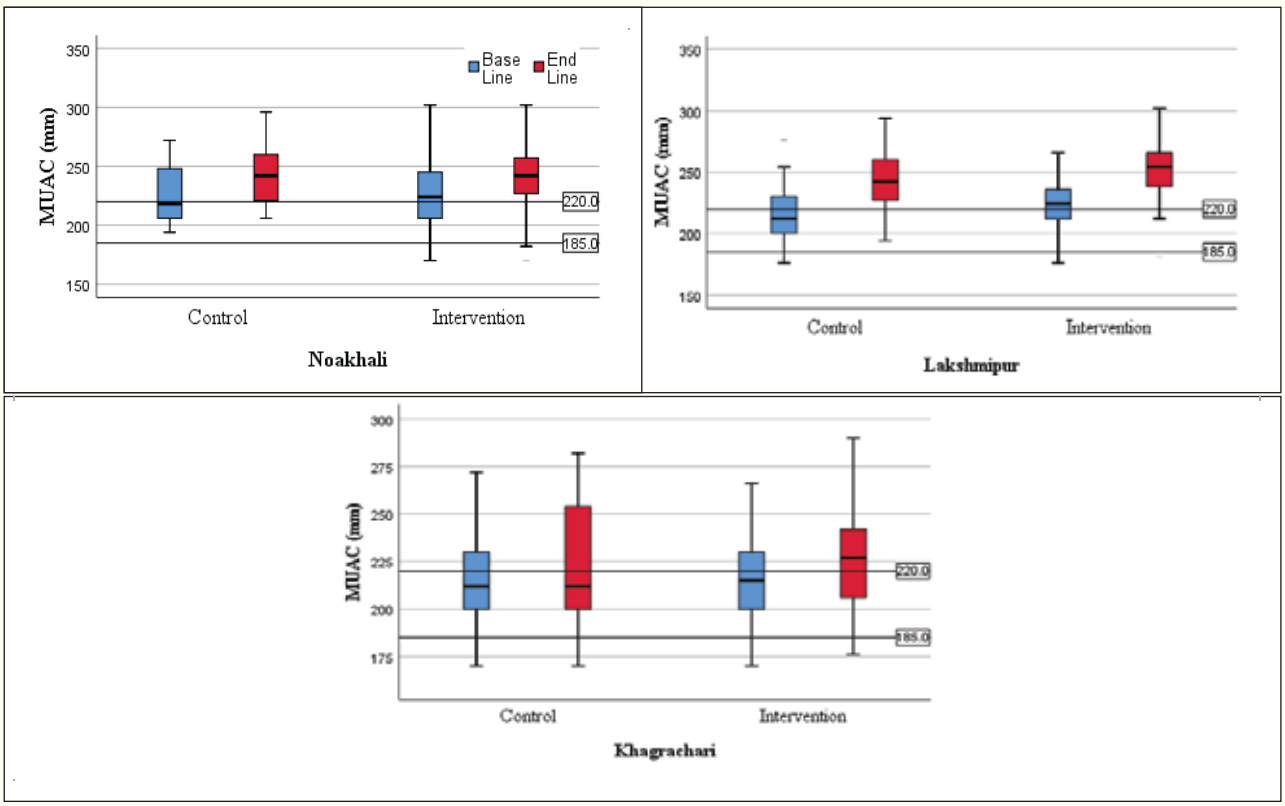


Fig 24. MUAC variability between two phases among the sampling areas with intervention

BMI (Body-mass index)

Except for the baseline of the Khagrachari intervention group, the mean BMI value of selected adolescent girls was within the normal range (18.5-24.9) established by WHO (WHO, 2022). Furthermore, almost all of the study groups had a low BMI level, particularly at the start. The value increased after the intervention ended in three different study locations when compared to the control. The average BMI in Noakhali (intervention and control) was higher than in the rest of the study area.

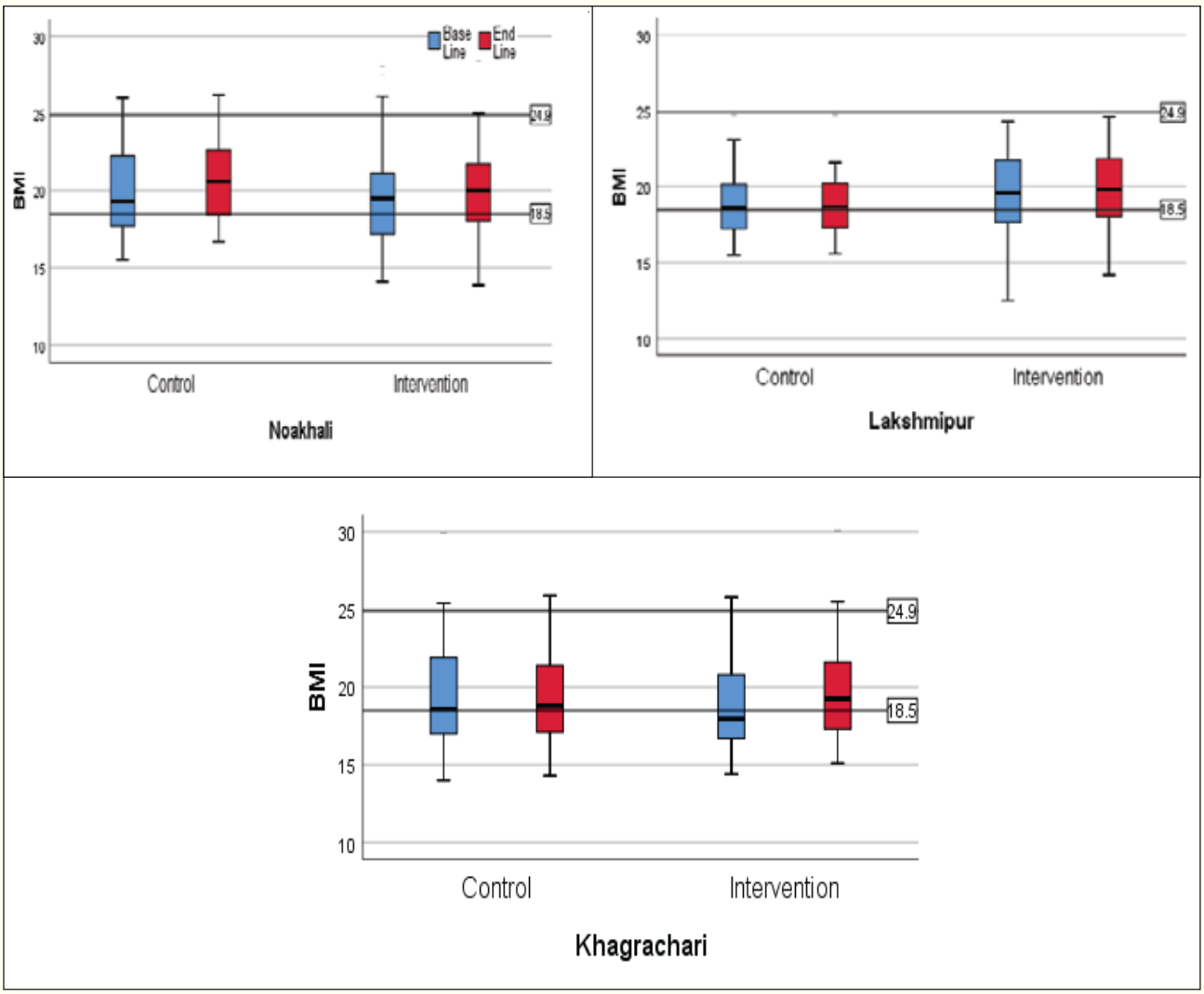


Fig 25. BMI variability between two phases among the sampling areas with intervention

Water Iron (Fe) in well

Iron levels were taken as supportive information to understand its state of concentration in tube-well water. Less than 0.3 mg/L iron in water is acceptable for humans. Except for Khagrachari (control), It was found that a significant number of tube wells were contaminated high iron content (>0.3 mg/L) exceeded normal ranges.

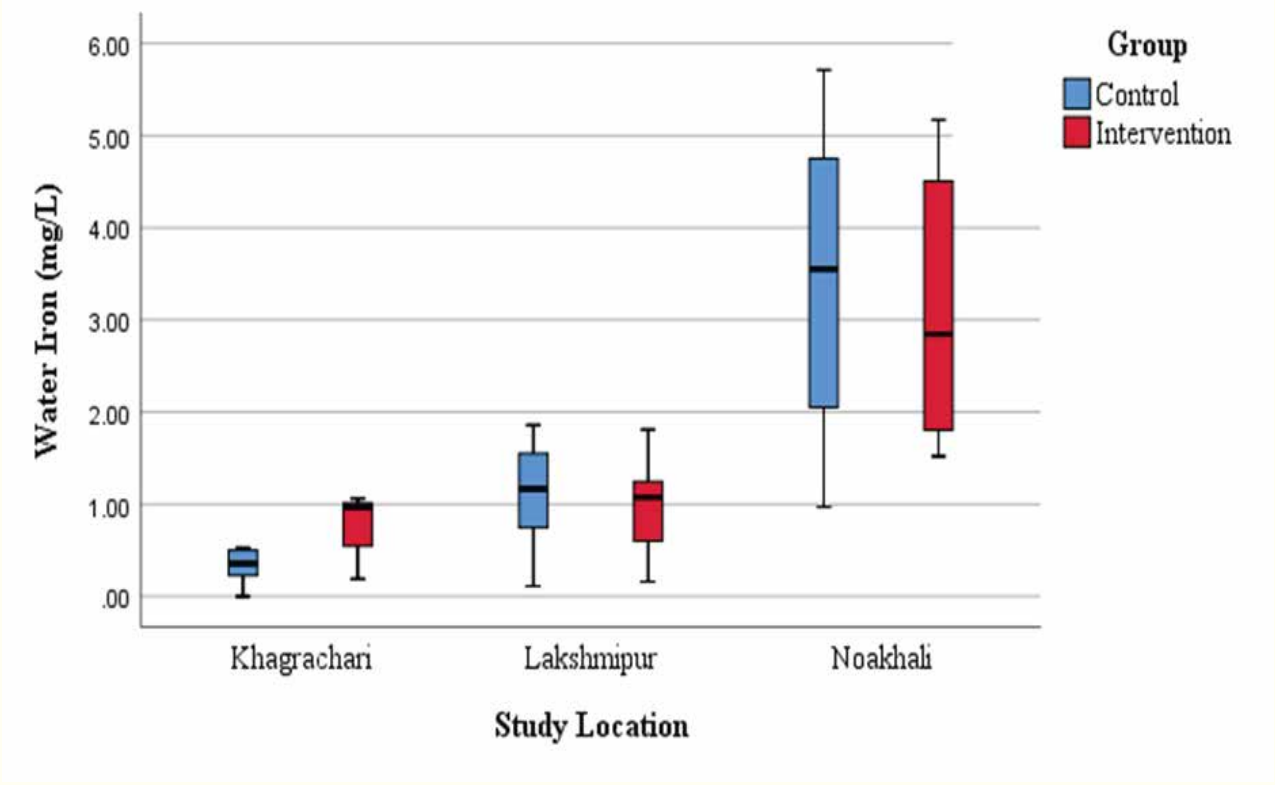


Fig 26. Iron level of well water of different study areas
(Nutrient data with mean (±SD))

14 days of food patterns

There were no missing days for cereals. Vegetable's consumption was higher during 2nd phases. Fish intakes were higher in Noakhali (Control & intervention).

Table 20. Consumption time frequency of foods during 14 days surveys

Area	Phases	Type	Fruits	Egg	Milk	Meat	Fish	Pulse	Vegetables	Leafy-veg	Cereals	Others
Khagrachari	Control	1st Phase	8.4	5	2.3	2.3	6.4	5.1	8.8	6	14	8.7
		2nd Phase	9.5	4.9	3.6	2.6	7.2	4	10.7	3.5	14	9.8
	Intervention	1st Phase	6.7	4	2.5	1.8	6.9	4.9	9.9	5.5	14	9.4
		2nd Phase	8.5	4.1	3.8	2.8	7.3	4.2	11.4	5.1	14	9.9
Lakshmipur	Control	1st Phase	3.9	6	1.4	2.3	7	8.9	9.2	5.5	14	4
		2nd Phase	4.3	4.6	1.9	2.4	8.3	6.3	9.3	4.3	14	7.1
	Intervention	1st Phase	3.6	5.8	2.1	2.3	7.8	8.7	8.6	5.8	14	3.9
		2nd Phase	5.6	5.1	1.9	2.3	9.2	6.5	9.8	5.8	14	7.2
Noakhali	Control	1st Phase	5.8	6.9	4.6	3.4	10.2	7.3	10.6	4.9	14	8.4
		2nd Phase	6.8	7.4	4.6	3.6	10	4.4	10.2	4.7	14	9.3
	Intervention	1st Phase	4.9	6.8	4.7	3.7	10.3	6.3	9.3	5.9	14	5.7
		2nd Phase	5.8	7.2	4.7	3.3	10.8	5.1	10.9	5.3	14	10.4

11.1.6. In-vivo model fish powder

It was found that serum Vit B12 and calcium conc. level increases approximately 10-20% due to consumption of Fish powder supplement by the mice. Moreover, Ferritin level was also increased significantly (approximately 40-50%, $p<0.05$) in the mice serum after providing Fish powder supplements.

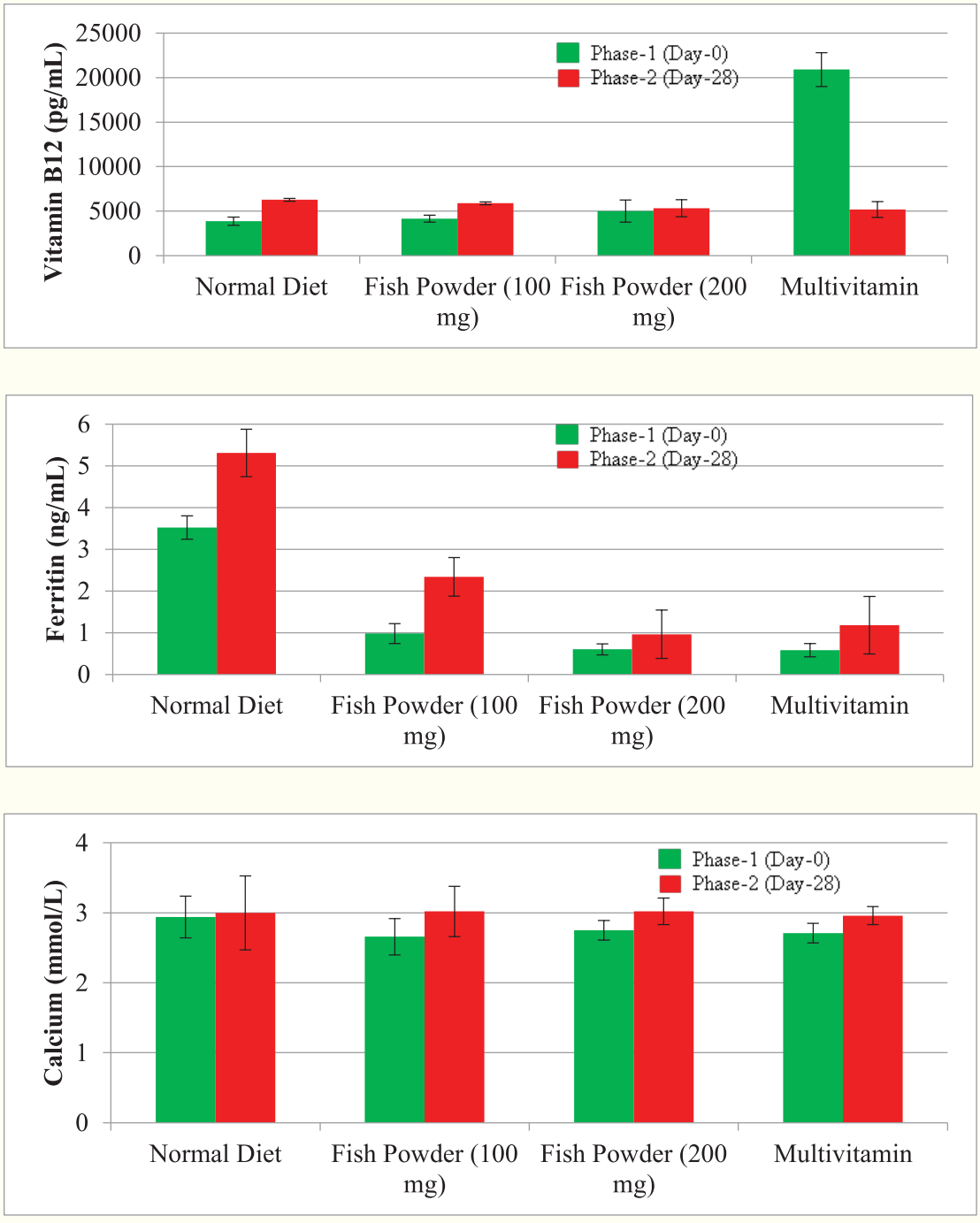


Fig 27. Biomarker's fluctuations in serums of mice

No changes in CRP and Iron level found due to introduction of Fish powder to the feed plan of anemic mice.

11.1.7. Responses of consumers on Fish powders' features

11.1.7.1. Fish powders survey related statistics

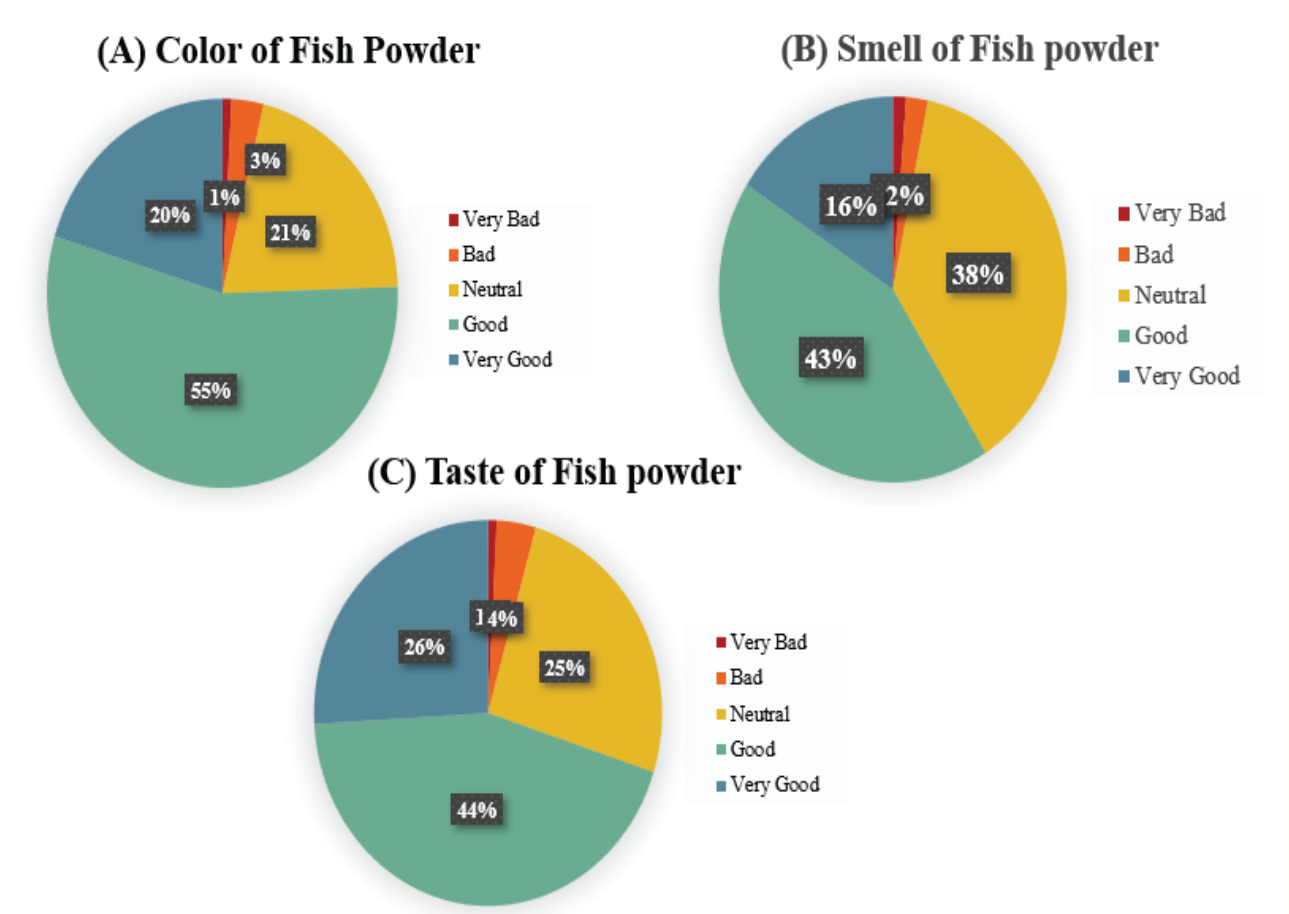


Fig 28. Responses of consumers on Fish powders' features, i.e., color (A), smell (B) and test (C)

Fig 28 presents that smell, taste and color of the product is very satisfactory to the respondents. According to the survey more than sixty percent respondents are happy of the taste, color and smell of Fish power consisting with Mitra et al. 2021.

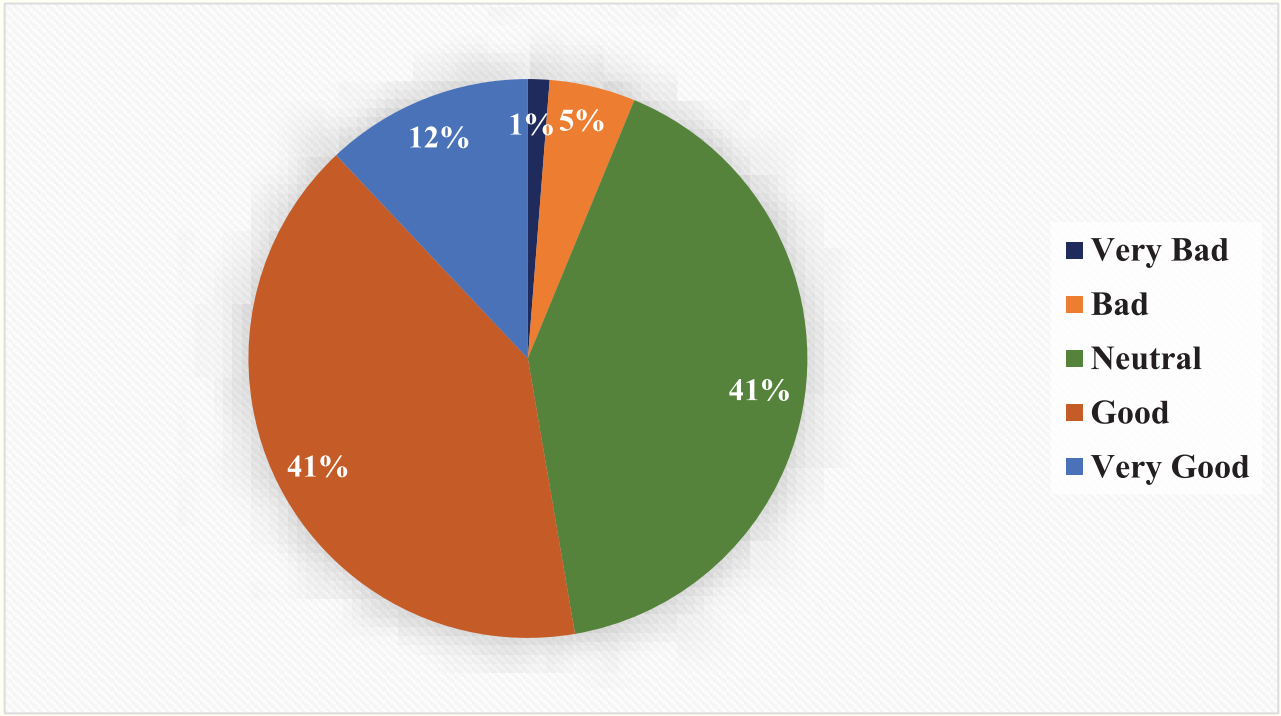


Fig 29. Fish powder taste in comparing with other cooked fish

Fig 29 presenting the comparative results between Fish power taste with cooked fish. Forty percent one percent respondents are saying good while same respondents are neutral of the question. We can conclude that most of the respondents are very positive of the Fish power.

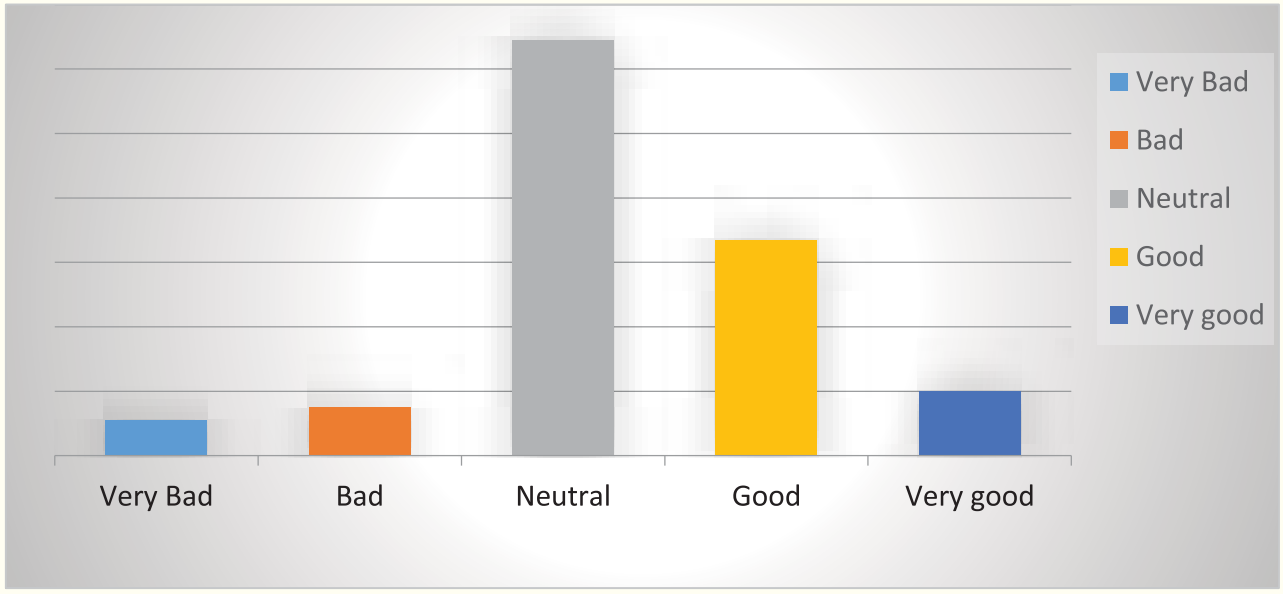


Fig 30. Consumption of Fish powder

Fig 30. describes the increasing nature of Fish power consumption of the respondents during the survey. The result indicates that overall consumption of fish power is increasing. It also presents that about forty percent respondents are like to consume Fish power instead of other cooked fish.

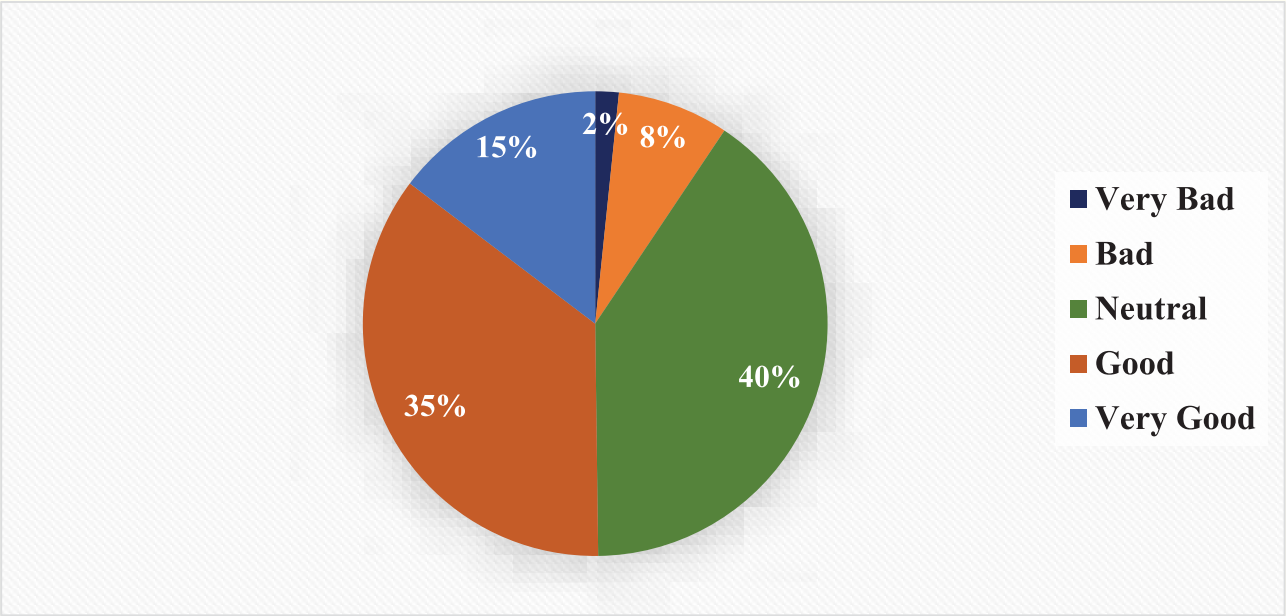


Fig 31. Overall satisfaction level considering color, smell and taste

Fig 31. entails that majority of the respondents are very satisfied of the Fish power considering taste, smell and color. Interestingly, fifty percent respondents in the survey pointed that they are very happy to use Fish powder as a new product in the market. Our result is consistent with Mirta et al. 2021

11.1.8. Fish powder time-related statistics

Data on the amount of time spent purchasing fish from the market, chopping it into pieces, and cooking the fish were collected from 172 respondents. It is estimated that it takes an average of 86.39 minutes to cut, wash, and cook one kilogram of Chapila fish and that it takes an average of 50 minutes after purchasing the fish to begin the cooking process. On the other hand, consumers who used Fish power did not need to spend additional time or money after washing and cutting the fish into pieces, and it also did not require additional time to purchase consistently. because fish powder can be kept for a relatively long time even after being opened. Instead, responsible members of the household might considerably contribute to the household in other productive activities by expanding this time into other meaningful uses. According to the survey results, it is simple for us to assert that Fish powder saves time and lowers the tension caused by work, both of which will have significant positive effects on the economy.

11.1.9. Fish powder safety related statistics

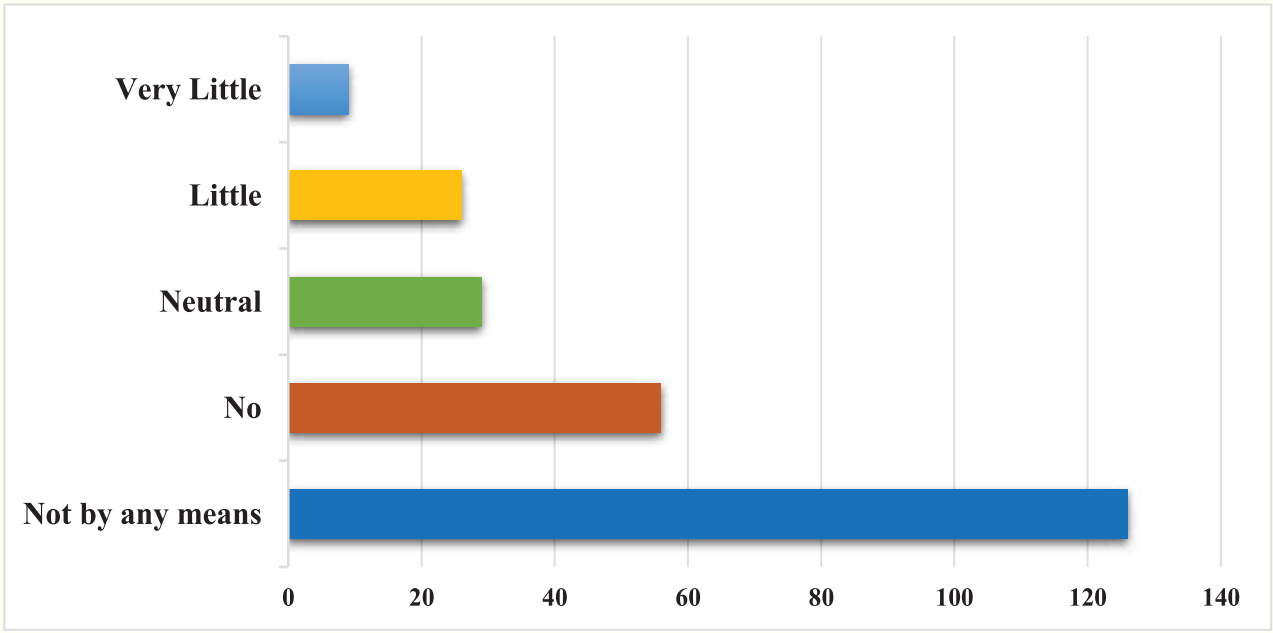


Fig 32. Facing any problem after eating cooked Fish Powder

Reactions from the respondents after eating Fish powder are shown in Fig 32. Findings rteflects thatt most of the respondents didn't find any physical and mental sickness (head ache, tummy ache, vomiting etc.) by eating this powder consisting with Hoque and Myrlan 2022

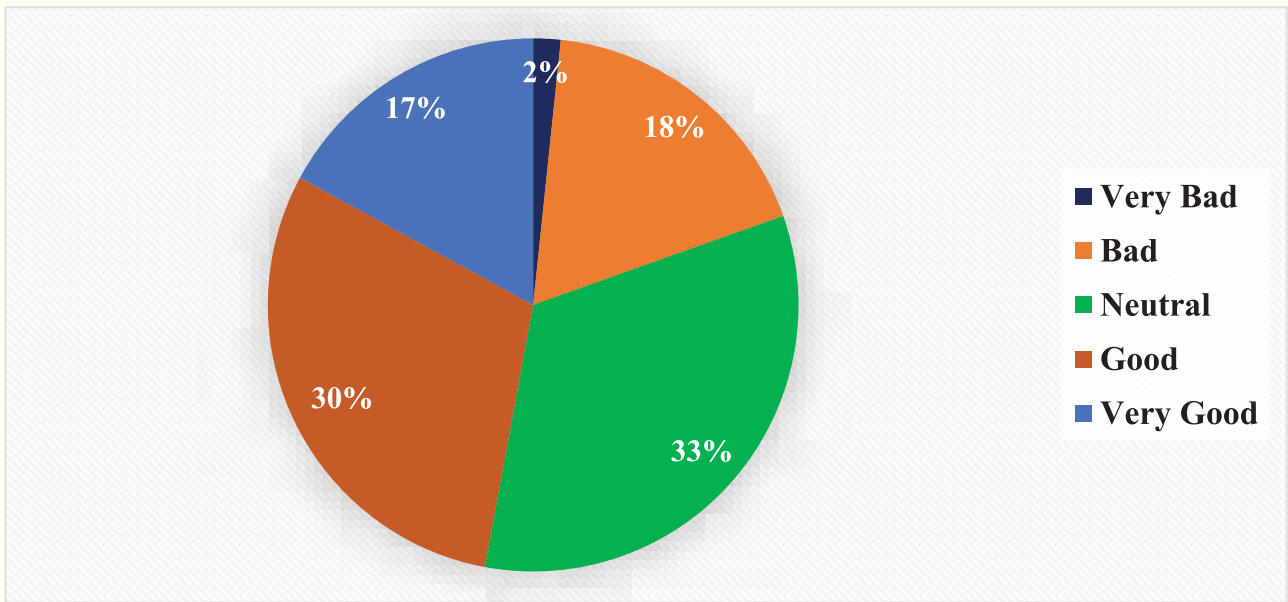


Fig 33. Fish powder safety to the children

Fig 33 describes the safety of the Fish powder to the children while majority of the respondents answer is positive and they like to give to their children comparing to Hoque and Myrlan 2022.

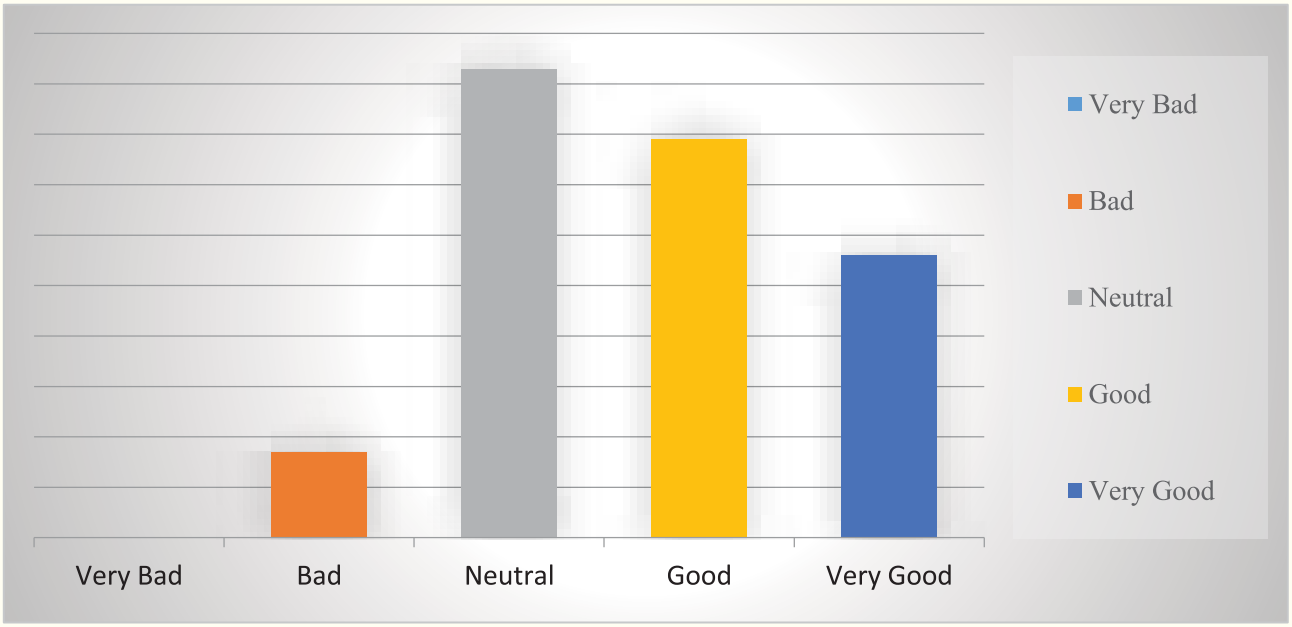


Fig 34. Fish powder safety compared to dry fish

Fig 34 represents the perception level between Fish powder and dry fish from the respondents. The results say most of the respondents like to take more Fish power than dry fish. The result shows that Fish powder is extremely safe compared to dry fish (Hoque and Myrlan 2022).

Fig 35. Fish powder safety compared to raw fish

Fig 35 represents the perception level between Fish powder and raw fish from the respondents. The results express that most of the respondents like to believe that Fish power is very useful compare to dry fish. The result shows that Fish powder is extremely safe compared to dry fish.

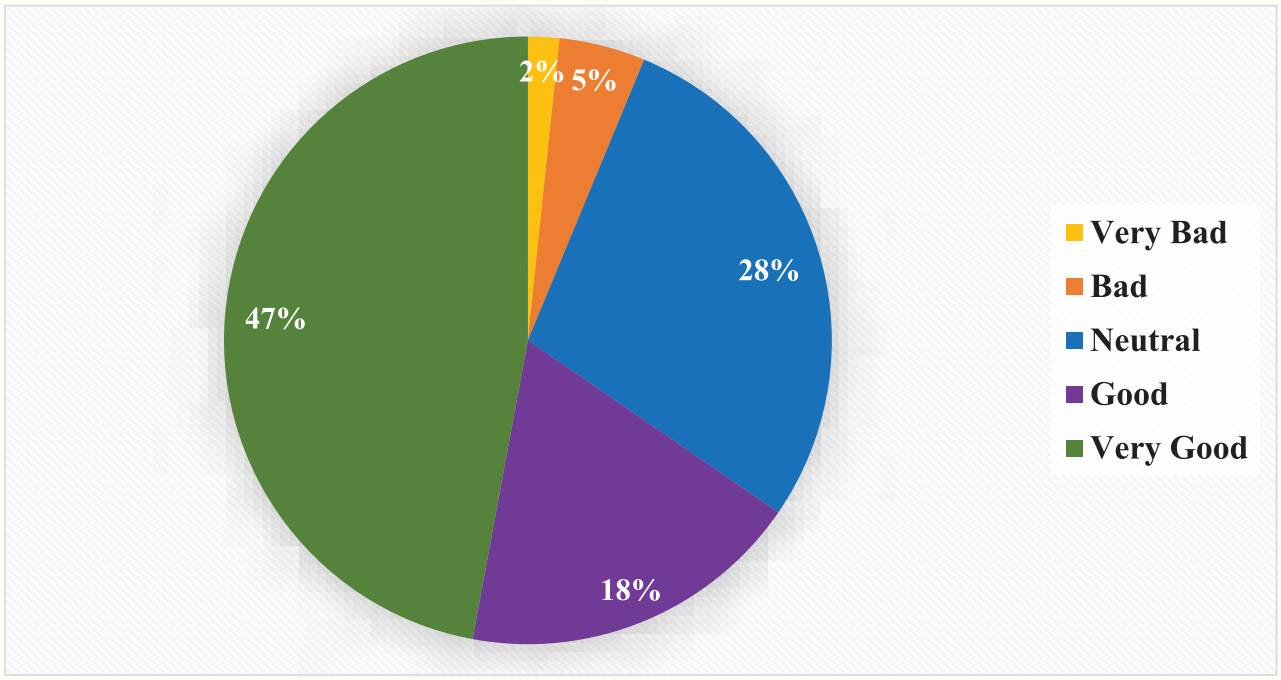


Fig 36. Fish powder role in the food safety

More than 70% respondents view the Fish powder product have a significant role in ensuring food safety of our country.

11.1.10. Fish powder nutrition related statistics

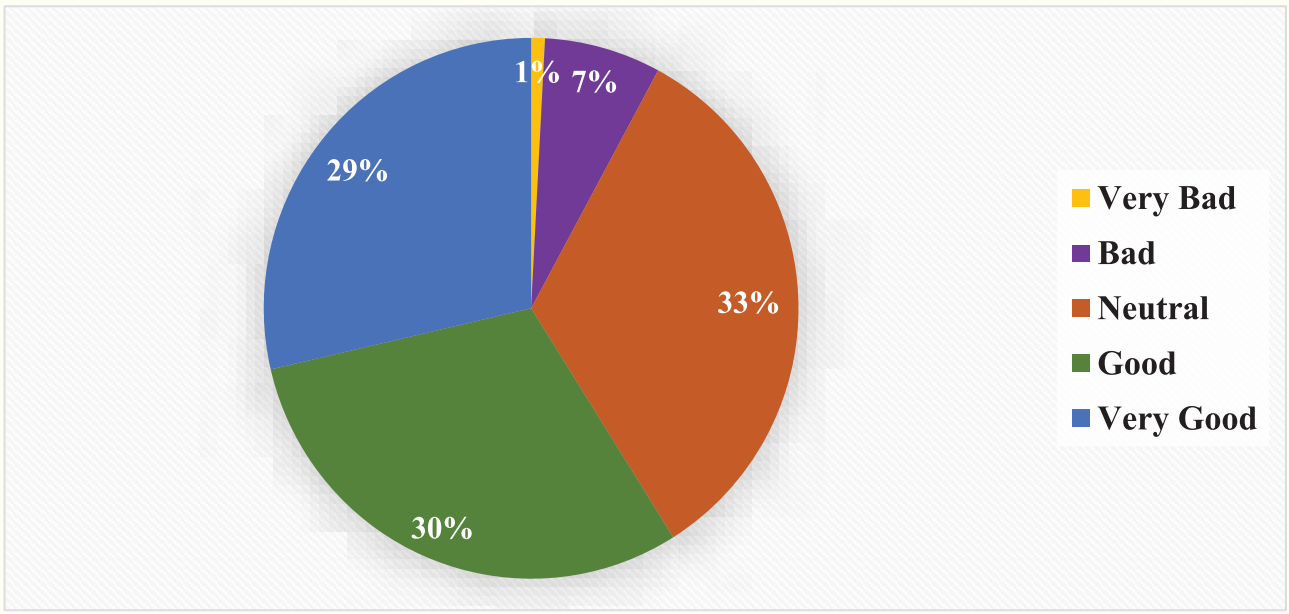


Fig 37. Nutrition of Fish powder and raw fish

Fig 37 presents the nutrition value level between Fish powder and cooked fish. According to the survey more than sixty percent respondents viewed that the Fish powder is a very good alternative source of nutrition compared to cooked fish meal nutrition.

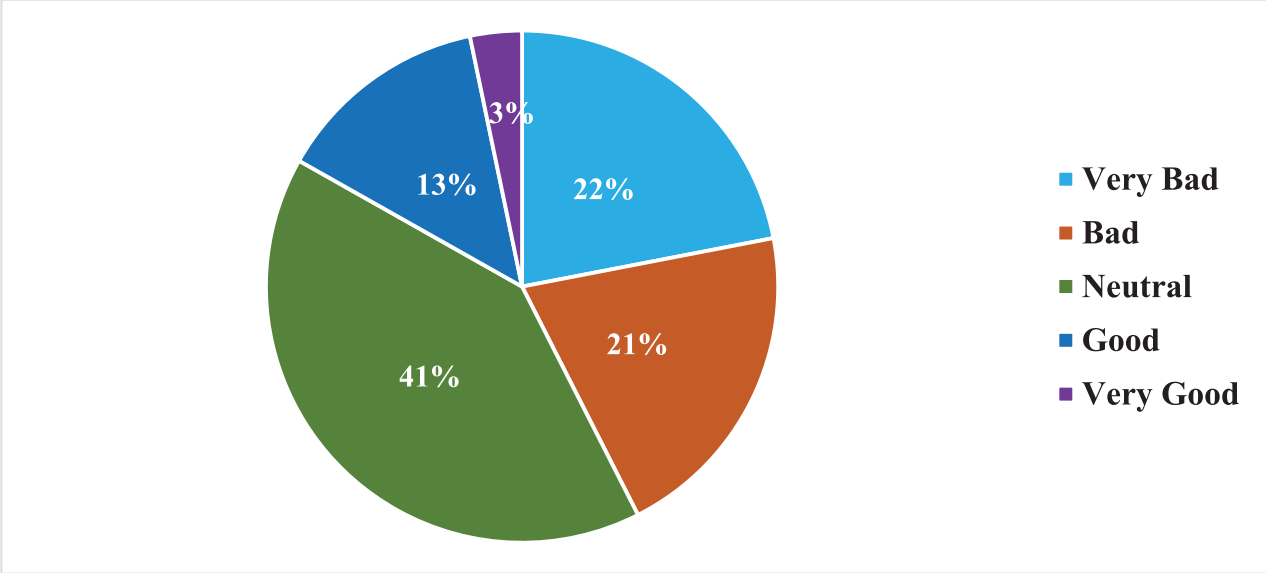


Fig 38. Role of Fish powder to avoid discriminating meal distribution

Fig 38 entails how food power could help to stop discriminating behavior in the family members to distribute meals. More than 50% of respondents opined that the use of Fish powder could be an appropriate tool to curve nutritional discrimination against gender.

11.1.11. Fish powder marketing related statistics

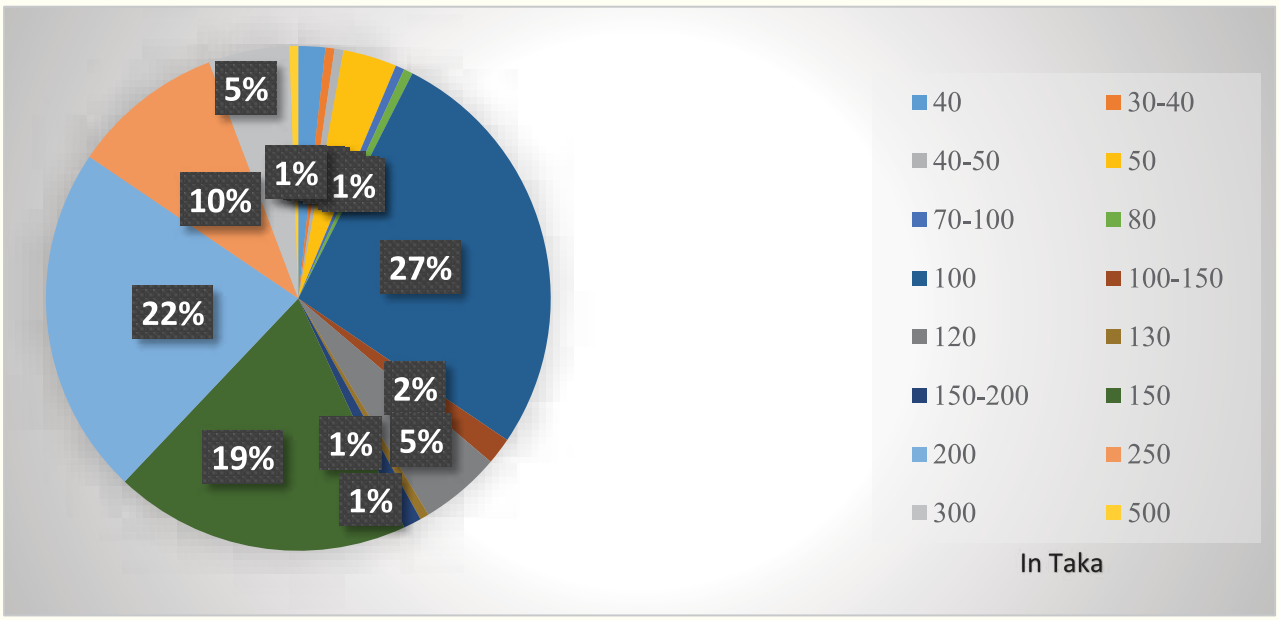


Fig 39. Willingness to pay for Fish powder

Fig 39 shows that majority of the respondents like to pay 100 to 150 take to buy Fish powder from the market.

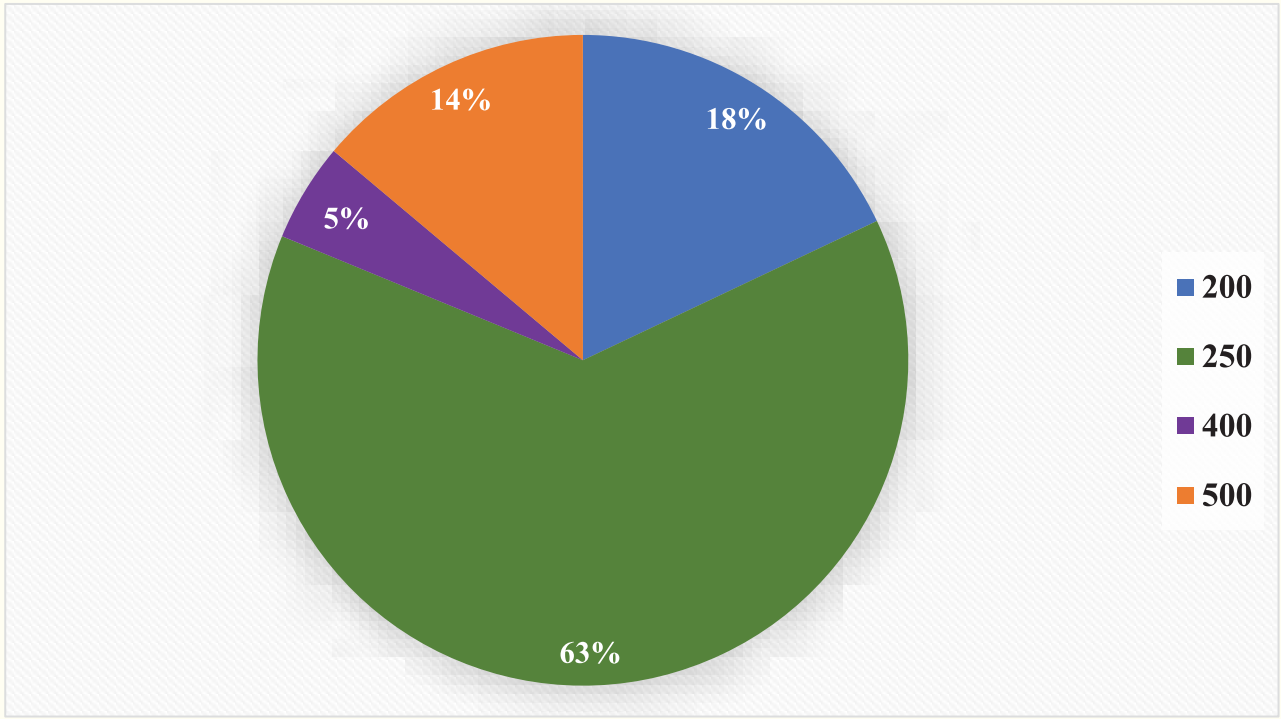


Fig 40. Willingness to buy Fish powder quantity

Fig 40 presents how much Fish powder they like to buy in a packet. Most of the respondents reply that they want to busy 250 g of Fish powder at a time.

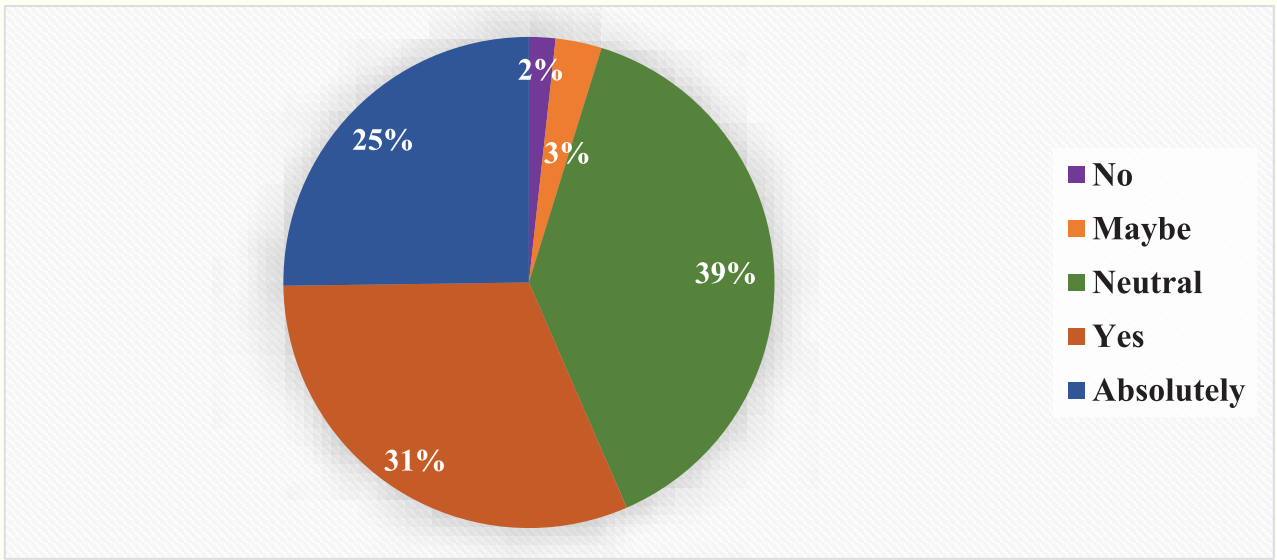


Fig 41. Like to buy Fish powder

Fig 41 presents whether the respondents like to buy the product. The result is very satisfactory and showing that more than ninety percent respondents want to buy Fish powder without any hesitation.

11.1.12. SWOT analysis of Fish powder

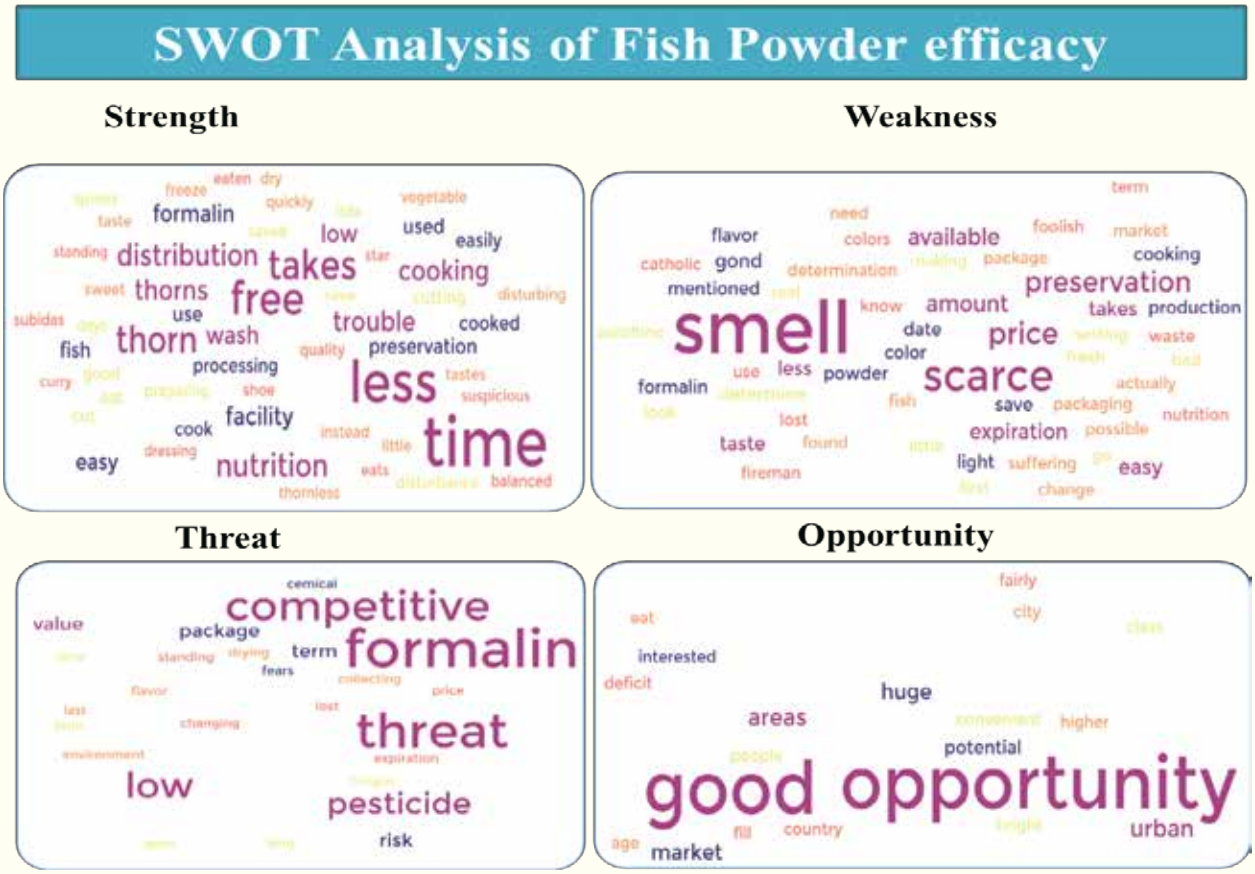


Fig 42. SWOT analysis in world cloud depiction

The word clouding SWOT analysis is presentation the most effective performance of Fish powder from the respondents.

Table 21. Five common cloud word of SWOT analysis

Strength	Weakness	Opportunities	Threats
Less time to cook	Smell	Huge demand	Competitive
Thorn less	Expiration	Potential markets	Formalin
Nutritious	Color	Urban areas	Pesticide
Easy cooking	Scarce	Good opportunity	Fungus

Table 22. Item cooked with Fish powder

Potato curry	Mashed shutki
Kafta	Barrow curry
Shrimp curry	Lati curry
Fish fry	Pui leaf
Cutlets	Small fish curry

11.1.13. Survey on product taste, preferences, durability, safety and marketing issues

In this survey method data was collected from 100 families of Noakhali Municipality, Maidje. Respondents were given the sample of product to consume it within 10 days after that a self-prepared questionnaire were provided consisting 100 questions including product taste, preferences, durability, safety and marketing issues. Through the Pie charts we found that 50% people fully satisfied with the products color, smell and taste, whereas 40% people were neutral. 55% found Fish powder safer than dry fish and 47% of the respondents feel Fish powder is safe for the children. Now most importantly 56% of the respondents want this product and 37% is neutral, which is good. Most of the respondents close to 50% want the product at price range of 100 Tk. to 200 Tk. They want to buy the product at 250 g packet as quantity.

Plate 32. Some cooked items collected during surveys

Through the SWOT, the respondents feel that strength of the product is "less time to cook", "nutritious "," thorn less", "easy to cook" and weakness of the product can be its smell and price have a very good opportunity in urban areas and it can have threat of competition and formalin.

11.1.14. Shelf-life prediction of FP

11.1.14.1. Shelf-life of chapila Fish powder

The shelf life of Fish powder was determined by the Rule of Ten. where two different storage temperatures (45°C and 55°C) were executed during the experiment. TVB-N values of Fish powder were observed at 45°C and 55°C storage temperatures after 15, 30, and 45 days. Besides this, at both temperatures, different characteristics of Fish powder were observed. And finally, the shelf-life of Fish powder was determined as 1 year by studying the TVB-N values and another parameter of Fish powder at 55°C after 45 days later. So final product shelf-life is one year from production time. Following Table 23 represent the shelf-life of the product at different temperature compare with one-year aging equivalency.

Table 23. Accelerated aging equivalency

Degrees C	Degrees F	1-Year Shelf-Life Equivalency (Weeks)
35	95	26.07
40	104	18.03
45	113	13.03
55	131	6.51
60	140	4.61

Here,
Based on Q10 = 2
Ambient Temp. = 25°C

11.1.14.2. Summary of Fish powder shelf-life

i. Chemical features of Chapila Fish powder

The proximate composition, mineral content, pH, TVBN, and peroxide value are present in Table 24. The TVBN and peroxide values are increased gradually during the accelerated storage period. The fatty acid profile of Chapila Fish powder is shown in Table 25.

The shelf-life formulation used for chapila Fish powder

In the present study, the desired expiration date of the developed Fish powder is one year, and the test temperature was selected 45°C and 55°C, Q_{10} is 2; the accelerated aging time duration is determined as follows:

$A1 = 2^{((45-25)/10)} = 4$

$A2 = 2^{((55-25)/10)} = 8$

B1 = 365 days/4 = 91 days
B2 = 365 days/8 = 45 days

Table 24. Chemical features of Chapila Fish powder storage at 45°C and 55°C

Parameters	Packaging types	Storage periods			
		0 day	15 days	30 days	45 days
Moisture (%)	FPZP 45°C	11.72±0.02	6.64±0.82	5.14±0.32	4.59±0.67
	FPVP 45°C	10.01±0.06	7.14±0.43	5.56±0.17	4.88±0.46
	FPZP 55°C	12.56±0.23	2.97±0.19	2.31±0.53	2.08±0.20
	FPVP 55°C	9.99±1.06	2.94±0.41	2.27±0.46	2.02±0.75
Ash (%)	FPZP 45°C	21.95±1.09	23.15±2.14	23.81±1.45	24.03±0.78
	FPVP 45°C	22.66±2.06	22.72±1.57	23.78±1.08	24.21±2.33
	FPZP 55°C	21.81±3.25	22.46±4.62	23.67±2.24	23.89±2.51
	FPVP 55°C	22.6±2.02	23.71±3.47	23.83±2.04	24.21±3.09
Fat (%)	FPZP 45°C	9.34±0.43	7.42±0.44	7.06±0.06	6.65±0.21
	FPVP 45°C	10.47±0.25	7.63±0.36	7.15±0.26	6.83±0.44
	FPZP 55°C	11.03±1.36	10.13±0.24	9.49±0.16	9.02±1.43
	FPVP 55°C	8.32±1.33	7.03±1.13	6.92±0.02	6.22±1.07
Protein (%)	FPZP 45°C	56.59±3.41	64.52±6.65	66.04±2.27	61.12±2.27
	FPVP 45°C	62.93±1.09	66.74±1.81	67.73±2.53	64.34±7.46
	FPZP 55°C	60.03±4.41	64.65±5.29	67.63±2.41	63.43±7.65
	FPVP 55°C	55.13±3.24	67.51±5.17	69.96±1.19	66.11±5.33
Iron (mg/100g)	FPZP 45°C	6.28±0.07	7.38±1.07	8.99±0.23	9.23±0.44
	FPVP 45°C	4.63±0.08	8.24±0.04	9.04±0.75	9.45±0.54
	FPZP 55°C	8.3±0.61	8.47±0.09	8.98±0.97	9.34±1.67
	FPVP 55°C	7.76±1.73	8.81±1.04	9.28±2.69	9.88±0.54
Phosphorous (mg/100g)	FPZP 45°C	426.46±11.28	502.5±10.59	662.92±0.45	723±2.23
	FPVP 45°C	509.77±4.35	572.79±6.47	688.33±8.87	801±1.77
	FPZP 55°C	417.95±9.67	493.82±22.74	560.38±11.23	606.31±8.76
	FPVP 55°C	531.3±6.55	590.01±7.28	625.82±3.66	670.42±2.87
pH	FPZP 45°C	6.28±0.04	6.13±0.03	5.83±0.12	5.26±0.20
	FPVP 45°C	6.23±0.43	6.21±0.04	5.9±0.32	5.23±0.33
	FPZP 55°C	6.25±0.56	6.22±0.08	5.71±0.05	5.22±0.08
	FPVP 55°C	6.32±0.76	6.16±0.07	5.76±0.45	5.47±0.34
TVBN (mg N/100g)	FPZP 45°C	7.5±1.64	11.5±0.40	12.02±0.56	13.53±0.50
	FPVP 45°C	8.5±0.50	10.52±0.44	10.95±0.76	11.43±0.41
	FPZP 55°C	7.88±0.79	12.48±1.23	13.27±0.49	14.65±1.50
	FPVP 55°C	8.72±1.67	11.02±2.82	11.89±1.50	12.78±0.63
Peroxide value (meq/kg fat)	FPZP 45°C	3.15±0.87	7.65±1.22	9.28±0.98	13.34±1.09
	FPVP 45°C	3.05±0.34	5.23±0.56	7.21±1.03	10.45±0.77
	FPZP 55°C	3.23±0.34	8.66±1.05	11.27±1.18	15.74±2.01
	FPVP 55°C	3.09±0.45	6.73±0.72	9.21±1.21	12.22±1.37

FPZP= Fish powder zipper pack, FPVP=Fish powder vacuum pack

Table 25. Fatty acid profile of Chapila Fish powder storage at 45°C and 55°C

Fatty acid (%)	Packaging types	Storage periods			
		0 day	15 days	30 days	45 days
C14	FPZP 45°C	8.63±1.34	4.88±0.31	3.98±0.46	1.33±0.27
	FPVP 45°C	9.4±2.45	7.97±1.11	6.34±1.04	4.32±1.06
	FPZP 55°C	10.06±0.98	4.35±0.23	3.65±0.33	1.29±0.09
	FPVP 55°C	9.42±1.07	6.45±0.05	5.76±0.02	3.87±0.29
C16	FPZP 45°C	39.77±4.67	41.06±3.67	43.71±8.34	43.78±11.55
	FPVP 45°C	38.81±4.09	39.67±3.76	42.03±3.99	47.23±5.91
	FPZP 55°C	37.34±6.93	42.67±3.87	44.87±3.81	44.87±4.44
	FPVP 55°C	37.86±8.23	38.98±6.71	39.08±0.89	41.34±1.34
C18	FPZP 45°C	4.38±0.48	4.97±0.32	5.09±1.01	4.58±0.99
	FPVP 45°C	4.51±0.56	4.78±0.76	5.01±0.19	4.97±0.66
	FPZP 55°C	4.34±0.45	4.49±0.69	4.99±0.87	5.24±0.39
	FPVP 55°C	4.76±0.99	4.88±1.06	4.95±1.11	4.91±0.49
C18:1	FPZP 45°C	26.81±4.98	28.95±3.89	31.56±4.34	30.45±1.78
	FPVP 45°C	27.75±2.98	29.67±3.33	32.56±4.29	31.02±5.23
	FPZP 55°C	26.07±7.34	28.32±3.21	30.43±4.56	30.11±0.97
	FPVP 55°C	26.88±6.33	27.87±2.86	29.54±3.58	29.43±3.22
C18:2	FPZP 45°C	6.59±2.99	7.33±1.23	8.03±0.45	7.11±1.02
	FPVP 45°C	6.15±2.11	6.98±1.06	7.23±0.44	7.22±0.55
	FPZP 55°C	5.59±0.59	6.07±0.48	6.49±0.77	6.43±0.28
	FPVP 55°C	5.81±1.11	6.11±0.25	6.54±0.34	6.51±0.46
C18:3	FPZP 45°C	6.22±0.88	6.83±0.55	7.11±0.34	7.03±0.45
	FPVP 45°C	7.09±1.07	7.55±0.81	7.91±0.58	7.88±0.41
	FPZP 55°C	6.31±0.58	6.88±0.22	7.22±0.23	7.11±1.11
	FPVP 55°C	6.96±0.37	7.28±0.41	7.39±0.47	7.32±0.34
C20	FPZP 45°C	ND	ND	ND	ND
	FPVP 45°C	ND	ND	ND	ND
	FPZP 55°C	ND	ND	ND	ND
	FPVP 55°C	ND	ND	ND	ND
C22	FPZP 45°C	ND	ND	ND	ND
	FPVP 45°C	ND	ND	ND	ND
	FPZP 55°C	ND	ND	ND	ND
	FPVP 55°C	ND	ND	ND	ND
C22:1	FPZP 45°C	4.75±0.06	4.23±0.19	3.78±0.22	2.45±0.03
	FPVP 45°C	4.97±0.27	4.55±0.21	4.05±0.09	3.66±0.11
	FPZP 55°C	4.81±0.08	4.11±0.15	3.64±0.55	2.11±0.32
	FPVP 55°C	4.91±0.05	4.39±0.23	3.86±0.32	3.01±0.04
C24	FPZP 45°C	2.88±0.39	2.66±0.31	2.06±0.48	1.67±0.51
	FPVP 45°C	2.28±0.33	2.13±0.14	2.09±0.14	1.98±0.06
	FPZP 55°C	1.77±0.78	1.43±0.03	1.22±0.31	0.97±0.05
	FPVP 55°C	2.64±0.04	2.01±0.17	1.97±0.22	1.13±0.07

FPZP= Fish powder zipper pack, FPVP=Fish powder vacuum pack

Significant increments of TVBN values were noted after 30 days of storage. The highest increment was found by FPZP (55ºC) and, the lowest was FPVP (45ºC)

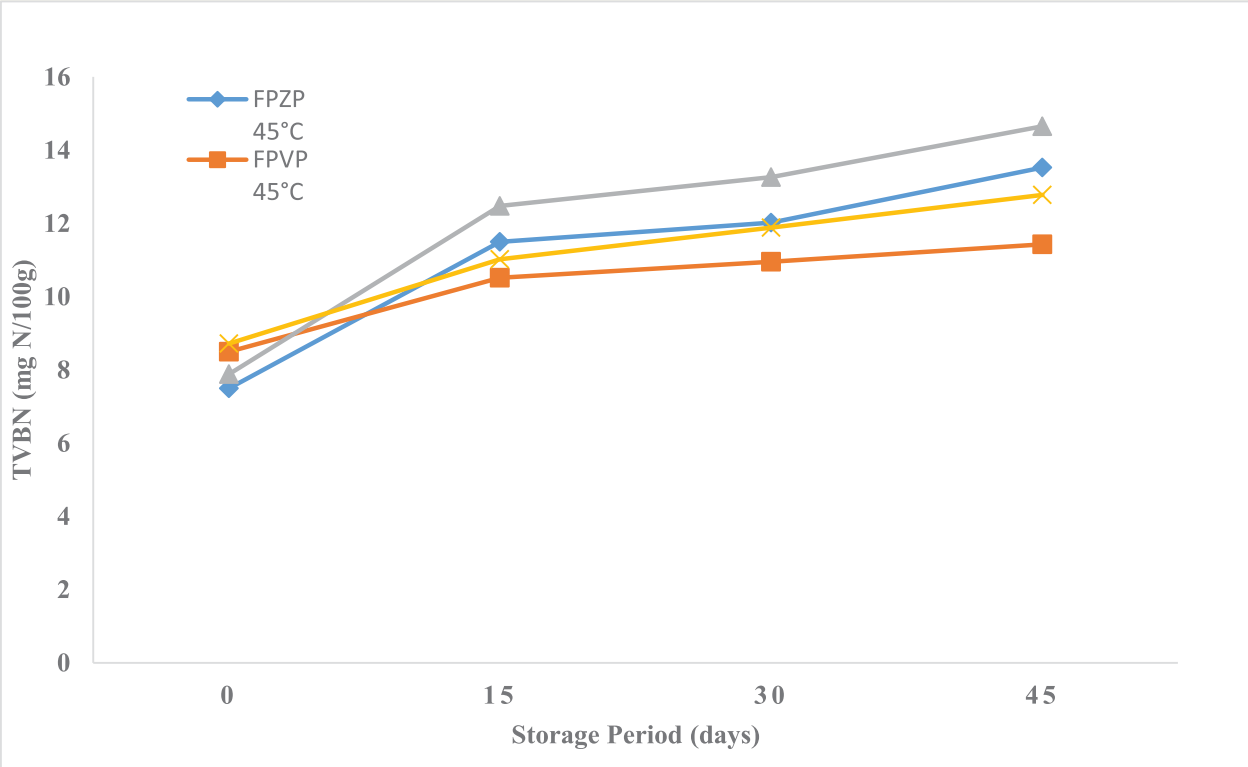


Fig 43. TVBN value in storage Chapila Fish powder

Significant increments of peroxide values were found after 15 days of storage. The highest increment was found by FPZP (55ºC) and the lowest was FPVP (45ºC)

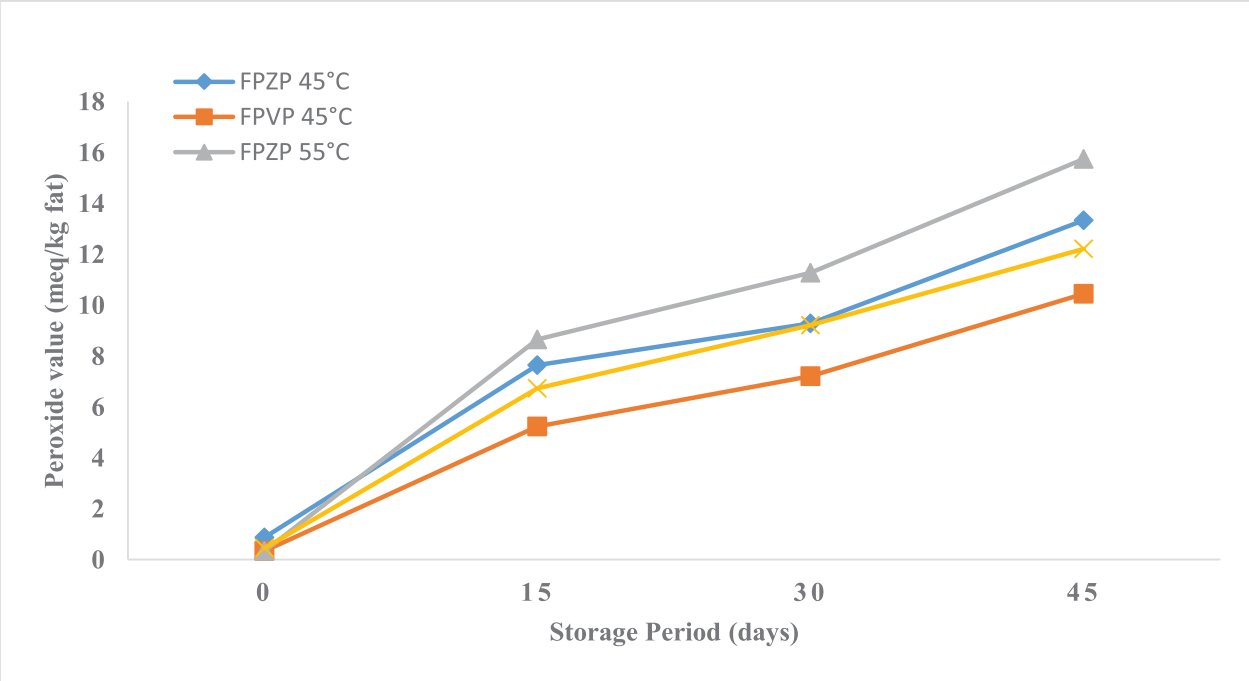


Fig 44. Peroxide value in storage Chapila Fish powder

ii. Shelf-life summaries

The lipid oxidation and degradation occurred in Chapila Fish powder during accelerated storage, which were reflected by the increase in all oxidation indices including peroxide value, TVBN, and PUFA percentage. By calculation, temperature co-efficient(Q_{10}) of shelf-life at 45°C and 55°C the actual accelerated aging time duration value were 91 days and 45 days, respectively which is demonstrated developed Chapila Fish powder shelf-life to be one year due to the peroxide value and TVBN value are expectable range during the accelerated storage periods. Furthermore, the present study reported Chapila Fish powder in zipper bag packaging is deteriorated faster than vacuum packaging as well as the deterioration rate is closer at 55°C than 45°C.

11.2 Component 1 (RU)

11.2.1 Baseline survey

The present report describes the present status on the availability of value-added fishery products in different superstores of Dhaka city. A total of 10 outlets managers from different retail superstores in Dhanmondi, Mirpur and Bashundhara in Dhaka city (such as Shwapno Super Shop, Meena Bazar, Nandan Mega Shop, Prince Bazar etc.) was surveyed with the structured questionnaire.

In the superstores, 90% consumers prefer to buy iced fish compared to 10% preferred frozen fish. All superstores' outlets kept Ready-to-cook (RTC) fish products mainly Fish finger, Fish sausage, Fish nuggets, Fish ball, Fish samosa, Fish stick and Fish burger. However, no Ready-to-eat (RTE) fish products was available in superstores. Imported value-added fishery products was available in 60% outlets. On the other hand, various value-added meat-based products were available such as Chicken ball, Chicken sausage, Chicken & beef burger, Chicken & beef samosa, Chicken nuggets, Chicken stick etc. Most of the consumers (70%) prefer 250g pack of value-added fish or meat products according to the managers. Around 40% stores contained refrigerated fish or meat products whereas 60% has no such products. The main reason of not having the refrigerated product was low demand (60%) and low shelf life (40%). If refrigerated products available, then 70% respondents said consumer will buy it, rest said not buy it. In that case, 30% responded that low shelf life is the main reason and 70% was not familiar with those types of products. Around 60% managers responded that frozen products sold mostly than that of refrigerated (40%) in those superstores. Most of the managers (80%) saw the possibilities of selling refrigerated value-added fishery products (Table 26). Therefore, there is a scope to produce packed refrigerated value-added fishery products which can be easily sold for a more extended period at refrigerated condition in the superstores.

Table 26. Present status of value-added fishery products in the superstores of Bangladesh (n=10)

Sl. No.	Variation	Category	Frequency	Percentage
1	Location	Dhanmondi	4	40.0
		Mirpur	3	30.0
		Bashundhara	3	30.0
2	Demanded fish	Iced	9	90.0
		Frozen	1	10.0
3	Ready to cook product	Yes	10	100.0
		No	0	0
4	Ready to eat product	Yes	0	0
		No	10	100.0
5	Imported value-added	Yes	6	60.0
	Product	No	4	40.0
6	Pack size	250	7	70.0
		500	1	10.0
		Others	2	20.0
7	Refrigerated value added	Yes	4	40.0
	product	No	6	60.0
8	No why	Short shelf life	4	40.0
		Low demand	6	60.0
9	If supply Then What	Yes	7	70.0
		No	3	30.0
10	No what	Short shelf-life	3	30.0
		Not familiar	7	70.0
11	Value-added products	Refrigerated	4	40.0
	mostly sold	Frozen	6	60.0
12	Marketing possibility	Yes	8	80.0
	Value added products	No	2	20.0

11.2.2. Development and quality evaluation of the Ready-to-cook (RTC) and Ready-to-eat (RTE) products

A total of 9 value-added fishery products has been developed optimizing the amounts of ingredients and processing parameters such as boiling time, steaming time, refrigeration time, drying time, soaking time. The RTC products were Tilapia Fish ball, Tuna Fish ball, Pangas Fish sausage, Mackerel Fish sausage, Battered & breaded Tilapia Fillet, Battered & breaded white Snapper Fillet, Sardine Fish marinades and RTE products were Tilapia Fish crackers and Tuna Fish crackers.

Proximate composition is very important to assess the nutritional quality of a products. The protein content of those 9 products were in the range of 11-19% where highest 19.32% protein found in Sardine Fish marinades and lowest in 10.93% in Tuna Fish crackers. The moisture contents were in the range of 3.52-67.98% where highest 67.98% moisture found in Sardine Fish marinades and lowest in 10.93% in Tuna Fish crackers. On the other hand, the carbohydrate contents were in the range of 4.11-51.43% where highest 51.43% carbohydrates found in Tuna Fish crackers and lowest in 4.111% in Sardine Fish marinades (Table 27).

Fish crackers contained very high amount of carbohydrate (51%) due to using more than 50% tapioca starch. Besides, due to drying, carbohydrate content in the Fish crackers increased proportionally. Similarly, higher amount of carbohydrate (64%) was present in Big head carp Fish crackers (Zzaman et al., 2017). To sum up, these products could be used as a protein-rich fishery product in the market.

Table 27. Nutritional quality of value-added fishery products

SL	Products	Moisture (%)	Ash (%)	Protein (%)	Fat (%)	Carbohydrates (%)	Energy (Kcal)
1	Fish ball-Tilapia	61.60	4.35	13.61	6.62	14.13	170.5
2	Fish ball-Tuna	61.02	4.28	12.77	6.12	15.65	168.8
3	Fish sausage-Pangas	51.02	4.28	12.00	1.90	31.03	189.2
4	Fish sausage-Mackerel	52.15	4.78	12.33	2.38	28.44	184.4
5	Battered & breaded Fish-Tilapia	61.26	4.28	12.09	3.26	19.29	154.9
6	Battered & breaded Fish-white Snapper	60.56	4.63	12.94	3.98	18.36	161.0
7	Fish marinades-Sardine	67.98	5.96	19.32	1.90	4.11	110.8
8	Fish crackers-Tilapia	4.39	11.93	11.03	22.00	50.66	444.8
9	Fish crackers-Tuna	3.52	11.77	10.93	22.35	51.43	450.6

11.2.3 Overall quality and shelf-life of Ready-to-cook (RTC) and Ready-to-eat (RTE) fish products under modified atmosphere packaging at different storage temperature

11.2.3.1 Tilapia Fish ball

Table 28. Changes in pH value of Tilapia Fish ball under modified atmosphere packaging stored at 4°C temperature

Treatments	Storage period (days)					
	0	4	8	12	16	20
Not sealed pack (control)	6.34±0.06^{a}	6.31±0.06^{a}	6.01±0.11^{a}	5.92±0.54^{a}	6.01±0.12^{a}	6.11±0.02^{b}
MAP-1 (50% CO_2 & 50% N_2)	6.34±0.06^{a}	5.86±0.23^{a}	6.06±0.02^{a}	5.79±0.31^{a}	5.65±0.22^{a}	5.51±0.38^{ab}
MAP-2 (40%CO_2, 30% N_2 & 30% O_2)	6.34±0.06^{a}	5.94±0.03^{a}	6.10±0.10^{a}	6.13±0.16^{a}	5.43±0.16^{a}	5.23±0.05^{a}
MAP-3 (50% CO_2 & 50% O_2)	6.34±0.06^{a}	6.03±0.04^{a}	6.24±0.02^{a}	6.26±0.06^{a}	5.64±0.12^{a}	5.02±0.15^{a}

The initial pH value of the present study was 6.34. Then, the pH values showed a decreasing trend with some fluctuations under all packaging conditions. Comparatively lower pH was observed in MAP samples after 12th day of storage than control sample during the storage period (Table 28). However, there were no significant ($p>0.05$) differences in pH values among all packaging conditions during the storage period. The appropriate post mortem pH level in fresh fish is typically 6.8~7.0 (Metin et al. 2001). The pH values of all samples were within the acceptable limit. The dropped in pH value in MAP with CO_2 sample possibly occurred as a result of the dissolution of CO_2 in muscle tissues (Ježek and Buchtová 2012). Moreover, the fish muscle surface absorbs CO_2, thus acidifying it with carbonic acid formation (Banks et al. 1980).

Table 29. Changes in TVBN value (mg/100g) of Tilapia Fish ball under modified atmosphere packaging stored at 4°C temperature

Treatments	Storage period (days)					
	0	4	8	12	16	20
Not sealed pack (control)	1.96±0.79^{a}	2.38±0.20^{a}	1.82±0.20^{a}	1.96±0.40^{a}	2.24±0..40^{a}	-
MAP-1 (50% CO_2 & 50% N_2)	1.96±0.79^{a}	1.82±0.20^{a}	1.40±0.79^{a}	1.54±0.20^{a}	1.54±0.20^{a}	2.66±0.20^{a}
MAP-2 (40%CO_2, 30% N_2 & 30% O_2)	1.96±0.79^{a}	1.68±0.40^{a}	1.26±0.20^{a}	1.40±0.40^{a}	1.68±0.40^{a}	2.52±0.79^{a}
MAP-3 (50% CO_2 & 50% O_2)	1.96±0.79^{a}	1.96±0.40^{a}	2.10±0.59^{a}	1.54±0.59^{a}	1.82±0.20^{a}	2.52±0.40^{a}

In order to predict bacterial spoilage of fish, TVB-N is a widely used parameter as an indicator. The TVB-N value of 30-35 mg N/100 g is generally considered a standard limit for chilled stored fish (Connell 1995). In the present study, the initial TVB-N value of Tilapia Fish ball was 1.96

mg/100g. The values almost gradually decreased until 8th days for control, MAP-1 & MAP-2 and 12th days for MAP-3 samples and then showed an increasing trend with some fluctuation under all packaging conditions (Table 29). Comparatively lower TVBN values were observed 12th days onwards in all samples than control sample. However, there were no significant ($p>0.05$) differences in TVBN values among three packaging conditions during the storage period. The gradual increase in TVB-N values at later stage during the storage time possibly occurred due to increased bacterial growth and presence of endogenous enzymes (Ruiz-Capillas and Moral 2001, Islami et al. 2015). This finding is similar to the earlier studies, where a correlation was detected between quality deterioration and grown TVB-N values of MA packaged seafood (Özogul et al. 2004, Nayma et al. 2020).

Table 30. Changes in TBARS (mg MDA/Kg fish) value of Tilapia Fish ball under modified atmosphere packaging stored at 4°C temperature

Treatments	Storage period (days)					
	0	4	8	12	16	20
Not sealed pack (control)	0.94±0.21^{a}	0.74±0.15^{b}	1.17±0.11^{a}	1.07±0.28^{ab}	1.18±0.03^{b}	
MAP-1 (50% CO_2 & 50% N_2)	0.94±0.21^{a}	0.40±0.01^{a}	0.94±0.02^{a}	0.94±0.04^{a}	0.72±0.02^{a}	1.58±0.29
MAP-2 (40%CO_2, 30% N_2 & 30% O_2)	0.94± 0.21^{a}	0.42±0.02^{a}	1.00±0.03^{a}	1.42±0.06^{b}	1.03±0.10^{b}	1.45±0.11
MAP-3 (50% CO_2 & 50% O_2)	0.94± 0.21^{a}	0.71±0.03^{b}	0.96±0.13^{a}	1.42±0.09^{b}	1.46±0.07^{c}	1.59±0.23

The preliminary value of TBARS was 0.94 mg MDA/kg Tilapia Fish ball. The TBARS gradually increased with time and fluctuation during the storage period (Table 30). However, significantly (p <0.05) lower TBARS were observed in MAP-1 samples on the 12th and 16th day of storage compared to the control and MAP-2 & 3 samples (Table 30). The absence of O_2 may have caused the oxidation of PUFAs to be delayed. Masniyom et al. (2013) and Alice et al. (2020) Masniyom et al. (2013) and Alice et al. (2020) both found that in the absence of O_2 vacuum and MA packaged Tilapia and Goonch fish had lower MDA compared to the control sample. The standard limit for TBARS was set as 2 mg MDA/kg fish, and beyond this limit, an unpleasant odor and taste can be formed (Connell 1995). The TBARS values did not exceed the acceptable limit (2 mg MDA/kg) during the storage period for all samples (Table 30).

Table 31. Changes in aerobic plate count (log CFU/g) of Tilapia Fish ball under modified atmosphere packaging stored at 4°C temperature

Treatments	Storage period (days)					
	0	4	8	12	16	20
Not sealed pack (control)	4.37±0.04^{a}	4.52±0.12^{a}	4.83±0.11^{a}	6.02±0.25^{a}	7.54±0.07^{c}	7.91±0.45^{a}
MAP-1 (50% CO_2 & 50% N_2)	4.37±0.04^{a}	4.67±0.02^{a}	5.93±0.01^{a}	5.96±0.28^{a}	6.76±0.03^{ab}	7.22±0.24^{a}
MAP-2 (40%CO_2, 30% N_2 & 30% O_2)	4.37±0.04^{a}	4.79±0.02^{a}	5.30±0.87^{a}	6.29±0.03^{a}	6.93±0.30^{bc}	7.17±0.09^{a}
MAP-3 (50% CO_2 & 50% O_2)	4.37±0.04^{a}	4.68±0.10^{a}	5.79±0.03^{a}	6.01±0.21^{a}	6.15±0.20^{a}	7.13±0.16^{a}

For the evaluation of the microbiological quality of a product as well as for shelf-life calculation, APC is an important indicator. The APC of RTC Tilapia Fish ball was 4.37 log CFU/g indicated an acceptable initial quality of Fish ball. The bacterial counts of 2 - 6 log CFU/g are considered acceptable of newly caught freshwater fishes (rainbow trout, tilapia, sea bass, and silver perch) (Gelman et al. 2001). The APC of Tilapia Fish ball gradually increased with time under all packaging conditions during the whole storage period. There was no significant difference (p >0.05) observed in APCs among all treated samples until the 12th day of storage (Table 31). Nevertheless, significantly (p <0.05) lower APCs were evident on the 16th day of storage in MAP-3 samples compared to control and other treated samples.

According to ICMSF (1986), the upper acceptable limit of aerobic plate counts (APC) for Ready-to-cook fishery products is 7 log CFU/g. In the current study, the APCs exceeded the 7 log CFU/g on approximately 14th day for control, 18th day for MAP-1, 17th day for MAP-2 and 20th day for MAP-3 sample (Fig 45).

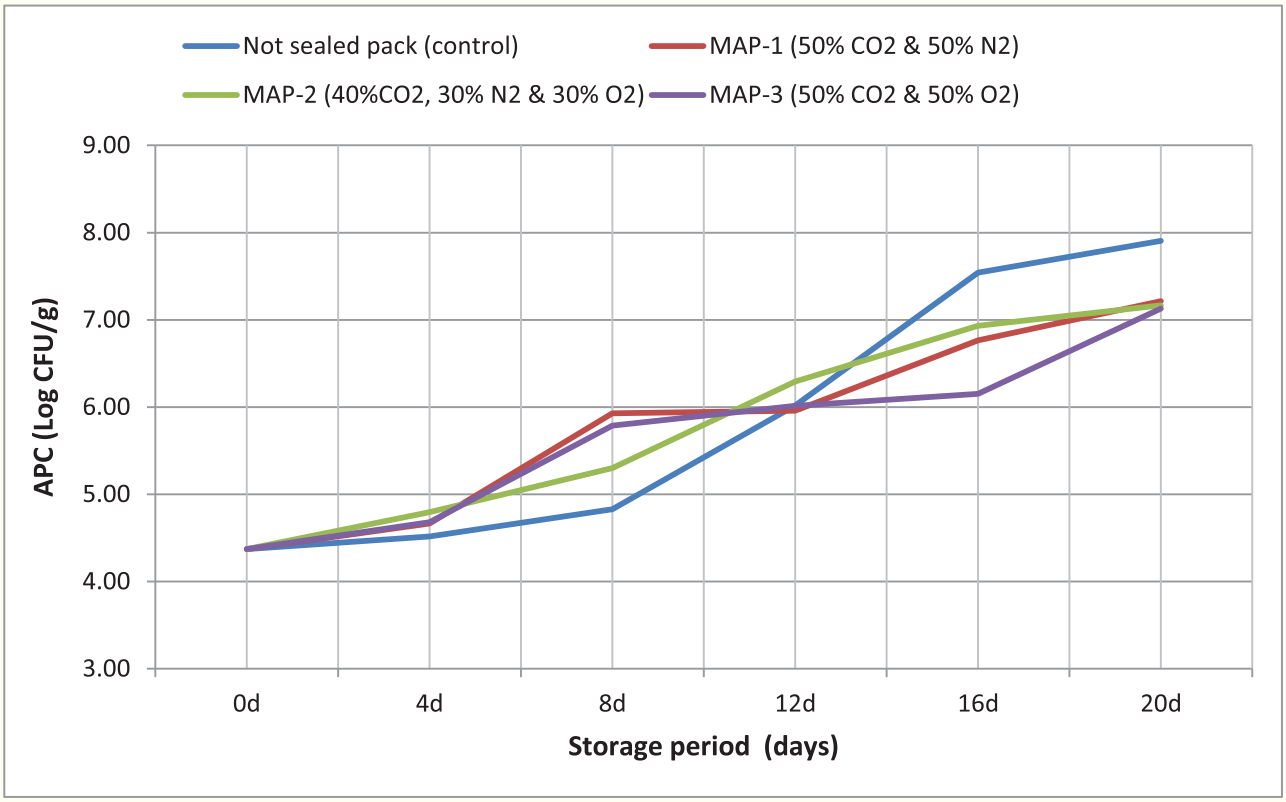


Fig 45. Changes in aerobic plate count (log CFU/g) of Tilapia Fish ball under modified atmosphere packaging stored at 4°C temperature

11.2.3.2 Tuna Fish ball

Table 32. Changes in pH value of Tuna Fish ball under modified atmosphere packaging stored at 4°C temperature

Treatments	Storage period (days)					
	0	4	8	12	16	20
Not sealed pack (control)	5.54±0.06^{a}	5.55±0.03^{a}	5.34±0.11^{a}	5.29±0.08^{a}	5.19±0.16^{a}	
MAP-1 (50% CO_2 & 50% N_2)	5.54±0.06^{a}	5.54±0.14^{a}	5.47±0.01^{a}	5.39±0.01^{a}	5.46±0.07^{ab}	5.31±0.32
MAP-2 (40%CO_2, 30% N_2 & 30% O_2)	5.54±0.06^{a}	5.60±0.01^{a}	5.39±0.00^{a}	5.41±0.06^{a}	5.39±0.03^{ab}	5.23±0.08
MAP-3 (50% CO_2 & 50% O_2)	5.54±0.06^{a}	5.57±0.11^{a}	5.40±0.01^{a}	5.45±0.01^{a}	5.58±0.05^{b}	5.48±0.06

The initial pH of the present study of Tuna Fish ball was 5.54. Then, the pH values showed a decreasing trend with some fluctuations under all packaging conditions. Comparatively lower pH was observed in control sample than other samples during the storage period (Table 32). However, there were no significant ($p>0.05$) differences in pH values among all packaging conditions during the storage period. The appropriate post mortem pH level in fresh fish is typically 6.8~7.0 (Metin et al. 2001). The pH values of all samples were within the acceptable limit. The dropped in pH value possibly occurred as a result of the dissolution of CO_2 in muscle tissues (Ježek and Buchtová 2012). Moreover, the fish muscle surface absorbs CO_2, thus get acidified with carbonic acid formation (Banks et al. 1980).

Table 33. Changes in TVBN value (mg/100g) of tuna Fish ball under modified atmosphere packaging stored at 4°C temperature

Treatments	Storage period (days)					
	0	4	8	12	16	20
Not sealed pack (control)	2.80±0.79^{a}	3.08±0.79^{a}	2.10±0.59^{a}	2.10±0.20^{b}	2.66±0.20^{a}	
MAP-1 (50% CO_2 & 50% N_2)	2.80±0.79^{a}	3.56±0.51^{a}	2.52±0.00^{a}	1.26±0.20^{a} b	1.68±1.19^{a}	2.66±0.59
MAP-2 (40%CO_2, 30% N_2 & 30% O_2)	2.80±0.79^{a}	1.96±0.40^{a}	1.68±0.40^{a}	1.12±0.00^{a} b	1.12±0.40^{a}	2.10±0.59
MAP-3 (50% CO_2 & 50% O_2)	2.80±0.79^{a}	1.45±0.20^{a}	2.24±0.40^{a}	0.84±0.40^{a}	1.54±0.20^{a}	1.54±0.59

The initial TVB-N value was 2.80 mg/100 g in Tuna Fish ball sample, and then fluctuated between 0.84 to 3.56. The TVBN values were in decreasing trend until 8th day for control sample and 12th day for other samples and then showed increasing trend (Table 33). However, there were no such significant ($p>0.05$) differences in TVBN values among all packaging conditions during the storage period (Table 35). The TVB-N value of 30-35 mg N/100 g is generally considered a standard limit for chilled stored fish (Connell 1995). TVBN values of all samples were within the acceptable limit during the entire storage period. The gradual increase in TVB-N values at later stage during the storage time possibly occurred due to increased bacterial growth and presence of endogenous enzymes (Ruiz-Capillas and Moral 2001, Islami et al. 2015). This finding is similar to the earlier studies, where a correlation was detected between quality deterioration and grown TVB-N values of MA packaged seafood (Özogul et al. 2004, Nayma et al. 2020).

Table 34. Changes in FFA (% of oleic acid) value of Tuna Fish ball under modified atmosphere packaging stored at 4°C temperature

Treatments	Storage period (days)					
	0	4	8	12	16	20
Not sealed pack (control)	0.25±0.05^{a}	0.25±0.05^{a}	0.25±0.05^{a}	0.32±0.05^{a}	0.42±0.10^{a}	
MAP-1 (50% CO_2 & 50% N_2)	0.25±0.05^{a}	0.32±0.05^{a}	0.32±0.05^{a}	0.39±0.05^{a}	0.32±0.05^{a}	0.35±0.10
MAP-2 (40%CO_2, 30% N_2 & 30% O_2)	0.25±0.05^{a}	0.28±0.10^{a}	0.25±0.05^{a}	0.32±0.05^{a}	0.32±0.05^{a}	0.25±0.05
MAP-3 (50% CO_2 & 50% O_2)	0.25±0.05^{a}	0.25±0.05^{a}	0.25±0.05^{a}	0.25±0.05^{a}	0.32±0.05^{a}	0.28±0.10

In the present study, the initial FFA value Tuna Fish ball was 0.25 % oleic acid and then fluctuated between 0.25 and 0.42 % oleic acid (Table 34). There were no significant ($p>0.05$) differences in FFA values among four packaging conditions during the storage period. As per quality specifications for crude fish oil, maximum acceptable values of 5% FFA were proposed (Bimbo, 1998). The results obtained in this study showed that the FFA content of Tuna ball did not reach the 5% limit during the storage period.

Table 35. Changes in TVARS (mg MDA/kg) of Tuna Fish ball under modified atmosphere packaging stored at 4°C temperature

Treatments	Storage period (days)					
	0	4	8	12	16	20
Not sealed pack (control)	5.78±1.03^{a}	15.38±0.79^{b}	14.64±0.48^{b}	14.08±5.84^{a}	12.70±0.30^{b}	
MAP-1 (50% CO_2 & 50% N_2)	5.78±1.03^{a}	9.39±0.20^{a}	13.16±0.40^{a}	10.25±0.84^{a}	9.76±0.52^{a}	12.97±0.28
MAP-2 (40%CO_2, 30% N_2 & 30% O_2)	5.78±1.03^{a}	16.47±3.37^{bc}	16.42±0.29^{c}	17.49±0.67^{a}	16.82±0.34^{c}	19.46±1.16
MAP-3 (50% CO_2 & 50% O_2)	5.78±1.03^{a}	20.47±0.25^{c}	20.25±0.23^{d}	16.35±0.37^{a}	16.62±0.15^{c}	7.20±0.16

The initial TBARS value was 5.78 mg MDA/kg tuna Fish ball. The TBARS gradually increased up to 8th day for MAP-1 sample and up to 4th day for other samples and kept almost steady in rest of the storage period (Table 35). However, significantly (p <0.05) lower TBARS were observed in MAP-1 samples in almost entire storage period compared to other samples. The absence of O_2 may have caused the oxidation of PUFAs to be delayed. This finding has been agreed by Masniyom et al. (2013) and Alice et al. (2020), who found that in the absence of O_2 vacuum and MA packaged Tilapia and goonch fish had the lower MDA compared to the control sample. The

standard limit for TBARS was set as 2 mg MDA/kg fish, and beyond this limit, an unpleasant odor and taste can be formed (Connell 1995). The TBARS values exceeded the acceptable limit (2 mg MDA/kg) in all samples during the storage period (Table 3). According to these results, it would be difficult to set limits for TBA levels, and TBA values may not give actual rate of lipid oxidation since malonaldehyde can interact with other components of fish such as nucleosides, nucleic acid, proteins and amino acids of phospholipids (Aubourg, 1993), and produce secondary metabolites that include carbohydrates, furfural, alkenals, alkadienals and other aldehydes and ketones (Botsoglou et al., 1994).

Table 36. Changes in aerobic plate count (log CFU/g) of Tuna Fish ball under modified atmosphere packaging stored at 4°C temperature

Treatments	Storage period (days)					
	0	4	8	12	16	20
Not sealed pack (control)	4.19 ± 0.13^{a}	4.59 ± 0.02^{a}	6.11 ± 0.05^{b}	7.73 ± 0.08^{b}	8.19 ± 0.12^{b}	
MAP-1 (50% CO_2 & 50% N_2)	4.19 ± 0.13^{a}	4.89 ± 0.20^{a}	6.27 ± 0.10^{b}	7.31 ± 0.12^{b}	7.10 ± 0.10^{a}	7.98 ± 0.24^{a}
MAP-2 (40%CO_2, 30% N_2 & 30% O_2)	4.19 ± 0.13^{a}	4.81 ± 0.17^{a}	5.87 ± 0.19^{b}	6.52 ± 0.32^{a}	7.20 ± 0.23^{a}	7.98 ± 0.26^{a}
MAP-3 (50% CO_2 & 50% O_2)	4.19 ± 0.13^{a}	4.34 ± 0.32^{a}	5.17 ± 0.02^{a}	6.22 ± 0.08^{a}	6.89 ± 0.03^{a}	7.56 ± 0.38^{a}

In Tuna Fish ball samples, the initial total viable count (TVC) was log 4.19 CFU/g. With the progression of storage, the APC values of Tuna Fish ball samples gradually increased in all packaging conditions. There were no significant ($p>0.05$) differences in APC observed amon g all packaging conditions until the 4th day of storage (Table 36). However, significantly ($p<0.05$) lower APC was observed on the 8th day and onwards in the MAP-3 (50% CO_2 & 50% O_2) sample compared to that of other samples (Table 36). The APCs exceeded the 7 log CFU/g, which is the acceptable limit (ICMSF, 1986) on approximately 10th day for control, 11th day for MAP-1, 14th day for MAP-2 and 16th day for MAP-3 sample (Fig 46).

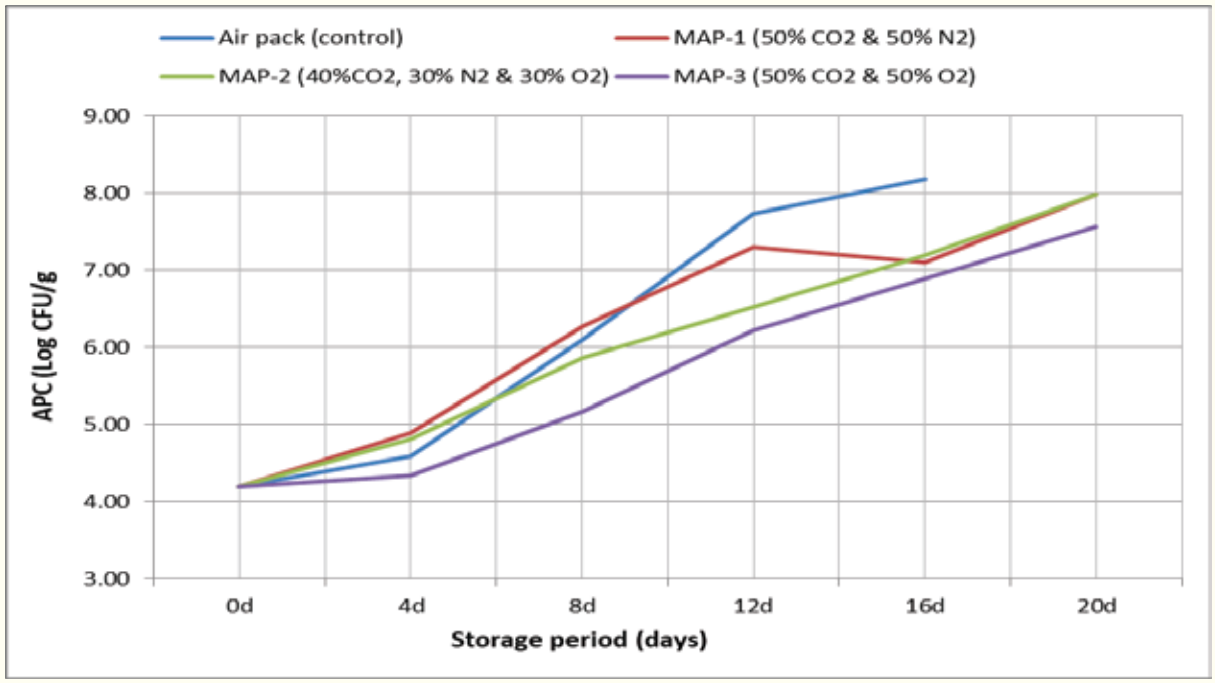


Fig 46. Changes in aerobic plate count (CFU/g) of Tuna Fish ball under modified atmosphere packaging stored at 4°C temperature

11.2.3.3 Pangas Fish sausage

Table 37. Changes in pH value of Pangas Fish sausage under modified atmosphere packaging stored at 4°C temperature

Treatments	Storage period (days)						
	0	5	10	15	20	25	30
Not sealed pack (control)	6.53±0.04^{a}	5.56±0.44^{a}	6.04±0.40^{a}	6.38±0.03^{a}	6.22±0.03^{a}	6.31±0.01^{a}	
MAP-1 (50% CO_2 & 50% N_2)	6.53±0.04^{a}	6.23±0.08^{a}	6.51±0.11^{a}	6.64±0.04^{b}	6.66±0.01^{b}	6.52±0.02^{a}	6.54±0.06
MAP-2 (40%CO_2, 30% N_2 & 30% O_2)	6.53±0.04^{a}	6.26±0.01^{a}	6.63±0.01^{a}	6.70±0..00^{b} ^{c}	6.66±0.03^{b}	6.52±0.16^{a}	6.45±0.07
MAP-3 (50% CO_2 & 50% O_2)	6.53±0.04^{a}	6.09±0.04^{a}	6.63±0.01^{a}	6.76±0.01^{c}	6.61±0.05^{b}	6.49±0.05^{a}	6.39±0.26

In the present study, it was observed that the initial pH value of Pangas Fish sausage 6.53 and then it decreased on 5th day and then gradually increased until 15th day and kept steady in rest of the storage period (Table 37). There were no significant ($p>0.05$) differences in pH values up to 10th day among all packaging conditions during the storage period. However, significantly ($p<0.05$) higher pH was observed on 15th & 20th day in all samples compared to that of control sample during the storage period (Table 39). The appropriate post mortem pH level in fresh fish is typically 6.8~7.0 (Metin et al. 2001). The pH values of all samples were within the acceptable limit.

Table 38. Changes in TVBN value (mg/100g) of Pangas Fish sausage under modified atmosphere packaging stored at 4°C temperature

Treatments	Storage period (days)						
	0	5	10	15	20	25	30
Not sealed pack (control)	1.54±0.20 a	1.40±0.79 a	2.52±0.79 a	2.66±0.59 a	2.46±0.08 a	2.94±0.20 a	
MAP-1 (50% CO_2 & 50% N_2)	1.54±0.20 a	2.66±0.20 a	2.24±0.40 a	2.24±0.40 a	2.54±0.20 a	3.08±0.79 a	3.64±0.40
MAP-2 (40%CO_2, 30% N_2 & 30%	1.54±0.20 a	2.24±0.00 a	2.38±0.59 a	1.82±0.20 a	2.62±0.31 a	2.94±0.59 a	2.94±0.20
MAP-3 (50% CO_2 & 50% O_2)	1.54±0.20 a	2.10±0.20 a	2.66±0.99 a	2.10±0.20 a	2.22±0.14 a	2.80±2.38 a	2.38±0.20

The initial TVB-N value was 1.54 mg/100 g in Pangas Fish sausage sample, and then fluctuated between 1.40 to 3.64 mg/100 g. The TVBN values gradually increased with time during the storage period with some fluctuations. However, there were no such significant ($p>0.05$) differences in TVBN values among all packaging conditions during the storage period (Table 38). The TVB-N value of 30-35 mg N/100 g is generally considered a standard limit for chilled stored fish (Connell 1995). TVBN values of all samples were within the acceptable limit during the entire storage period. The gradual increase in TVB-N values during the storage time possibly occurred due to increased bacterial growth and presence of endogenous enzymes (Ruiz-Capillas and Moral 2001, Islami et al. 2015). This finding is similar to the earlier studies, where a correlation was detected between quality deterioration and grown TVB-N values of MA packaged seafood (Özogul et al. 2004, Nayma et al. 2020).

Table 39. Changes in TBARS (mg MDA/Kg fish) value of Pangas Fish sausage under modified atmosphere packaging stored at 4°C temperature

Treatments	Storage period (days)						
	0	5	10	15	20	25	30
Not sealed pack (control)	3.01±0.09^a	3.10±1.03^a	2.84±0.05^a	3.14±0.32^a	3.18±0.38^a	3.78±0.12^a	
MAP-1 (50% CO_2 & 50% N_2)	3.01±0.09^a	3.13±0.01^a	2.81±0.23^a	4.29±0.48^a	4.29±0.11a b	4.33±0.60^a	5.15±0.48
MAP-2 (40%CO_2, 30% N_2 & 30% O_2)	3.01±0.09^a	3.46±0.01^a	2.90±0.27^a	4.13±0.05^a	4.89±0.57^b	4.18±0.74^a	4.96±0.11
MAP-3 (50% CO_2 & 50% O_2)	3.01±0.09^a	2.88±0.13^a	3.04±0.21^a	4.07±0.48^a	4.30±0.08^a b	4.56±0.07^a	5.70±0.05

The initial TBARS value was 3.01 mg MDA/kg Pangas Fish sausage. The TBARS values almost steady up to 10th day and then gradually increased in rest of the period in all packaging conditions (Table 39). There were no such significant ($p>0.05$) differences in TBARS values among four packaging conditions during the storage period. The absence of O_2 may have caused the oxidation of PUFAs to be delayed. This finding has been agreed by Masniyom et al. (2013) and Alice et al. (2020), who found that in the absence of O_2 vacuum and MA packaged tilapia and goonch fish had the lower MDA compared to the control sample. Nunes et al. (1992) reported that the acceptability limits of TBA value for fish stored in ice were 5–8 mg MA/kg flesh. The TBARS values of all packaging conditions were within the acceptable limit.

Table 40. Changes in aerobic plate count (log CFU/g) of Pangas Fish sausage under modified atmosphere packaging stored at 4°C temperature

Treatments	Storage period (days)						
	0	5	10	15	20	25	30
Not sealed pack (control)	4.51±0.10^{a}	4.60±0.14^{a}	4.51±0.11^{a}	7.00±0.18^{c}	7.48±0.05^{b}	7.63±0.19^{a}	
MAP-1 (50% CO_2 & 50% N_2)	4.51±0.10^{a}	4.68±0.34^{a}	4.99±0.10^{b}	5.87.26^{ab}	6.54±0.08^{a}	6.70±0.07^{a}	8.08±0.07
MAP-2 (40%CO_2, 30% N_2 & 30% O_2)	4.51±0.10^{a}	4.12±0.16^{a}	4.56±0.08^{a}	6.20±0.06^{b}	6.46±0.09^{a}	7.20±0.57^{a}	8.00±0..14
MAP-3 (50% CO_2 & 50% O_2)	4.51±0.10^{a}	4.22±0.15^{a}	4.46±0.06^{a}	5.33±0.25^{a}	6.27±0.05^{a}	7.00±0.13^{a}	8.07±0.01

The aerobic plate count (APC) of Pangas Fish sausage was 4.51 log CFU/g on plate count agar medium. The APC values of Pangas Fish sausage gradually increased with progression of time in all packaging conditions (Table 40). There were no significant ($p>0.05$) differences in APC observed among all packaging conditions until the 5th day of storage. However, significantly ($p<0.05$) lower APC was observed on the 20th day in all samples compared to control sample (Table 40). The APCs exceeded the 7 log CFU/g (ICMSF, 1986), which is the acceptable limit on approximately 15th day for control, 26th day for MAP-1, 23rd day for MAP-2 and 25th day for MAP-3 sample (Fig 47).

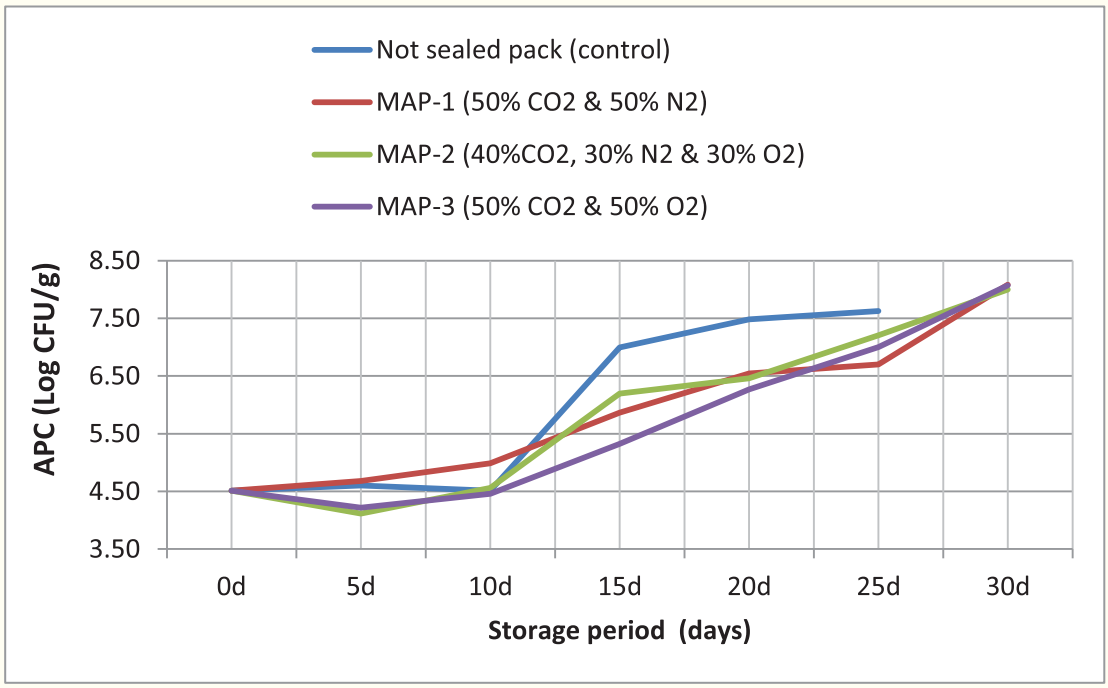


Fig 47. Changes in aerobic plate count (log CFU/g) of Pangas Fish sausage under modified atmosphere packaging stored at 4°C temperature

11.2.3.4 Mackerel Fish sausage

Table 41. Changes in pH value of Mackerel Fish sausage under modified atmosphere packaging stored at 4°C temperature

Treatments	Storage period (days)					
	0	7	13	17	21	31
Not sealed pack (control)	6.12±0.03^a	6.16±0.02^a	6.22±0.07^a	6.36±0.08^a	6.32±0.11^a	6.32±0.00^a
MAP-1 (50% CO_2 & 50% N_2)	6.12±0.03^a	6.17±0.01^a	6.27±0.05^a	6.36±0.06^a	6.35±0.01^a	6.35±0.00^a
MAP-2 (40%CO_2, 30% N_2 & 30% O_2)	6.12±0.03^a	6.17±0.00^a	6.29±0.01^a	6.37±0..04^a	6.37±0.01^a	6.33±0.01^a
MAP-3 (50% CO_2 & 50% O_2)	6.12±0.03^a	6.15±0.01^a	6.25±0.02^a	6.38±0.02^a	6.38±0.01^a	6.31±0.01^a

The initial pH of the present study of Mackerel Fish sausage was 6.12. Then, the pH values gradually increased up to 17th day and then kept remain steady during rest of the storage period. Comparatively lower pH was observed in control sample than other samples during the storage period (Table 41). However, there were no significant ($p>0.05$) differences in pH values among all packaging conditions during the storage period. The appropriate post mortem pH level in fresh fish is typically 6.8~7.0 (Metin et al. 2001). The pH values of all samples were within the acceptable limit. It was evident in the past study that the result of increased pH was linked with the generation of basic components such as ammonia, dimethylamine, trimethylamine, and other biogenic amines, as well as microbial spoilage (Goulas and Kontominas, 2007).

Table 42. Changes in TVBN value (mg/100g) of Mackerel Fish sausage under modified atmosphere packaging stored at 4°C temperature

Treatments	Storage period (days)					
	0	7	13	17	21	31
Not sealed pack (control)	1.82±0.20^a	3.64±1.58^a	2.24±0.00^a	2.66±0.59^a	2.66±0.20^a	3.21±0.18^a
MAP-1 (50% CO_2 & 50% N_2)	1.82±0.20^a	3.50±2.18^a	2.10±0.59^a	2.74±0.31^a	2.67±0.02^a	3.58±0.08^a
MAP-2 (40%CO_2, 30% N_2 & 30% O_2)	1.82±0.20^a	3.24±0.11^a	2.8±0.40^a	2.82±0.20^a	2.62±0.31^a	3.44±0.11^a
MAP-3 (50% CO_2 & 50% O_2)	1.82±0.20^a	3.64±0.40^a	2.38±0.20^a	2.60±0.51^a	2.60±0.07^a	3.80±0.96^a

The initial TVB-N value was 1.82 mg/100 g in Mackerel Fish sausage sample, and then fluctuated between 1.82 to 3.80 mg/100 g. The TVBN values increased on 7th day of storage and then decreased on 13th day of storage and then gradually increased in rest of the period in packaging conditions. However, there were no such significant ($p>0.05$) differences in TVBN values among all packaging conditions during the storage period (Table 44). The TVB-N value of 30-35 mg N/100 g is generally considered a standard limit for chilled stored fish (Connell 1995). TVBN values of all samples were within the acceptable limit during the entire storage period. The gradual increase in TVB-N values at later stage during the storage time possibly occurred due to increased bacterial growth and presence of endogenous enzymes (Ruiz-Capillas and Moral 2001, Islami et al. 2015). This finding is similar to the earlier studies, where a correlation was detected between quality deterioration and grown TVB-N values of MA packaged seafood (Özogul et al. 2004, Nayma et al. 2020).

Table 43. Changes in FFA (% of oleic acid) value of Mackerel Fish sausage under modified atmosphere packaging stored at 4°C temperature

Treatments	Storage period (days)					
	0	7	13	17	21	31
Not sealed pack (control)	0.53±0.05^a	0.35±0.10^a	0.32±0.05^a	0.28±0.10^a	0.35±0.00^a	0.46±0.15^a
MAP-1 (50% CO_2 & 50% N_2)	0.53±0.05^a	0.39±0.05^a	0.25±0.05^a	0.25±0.05^a	0.39±0.05^a	0.35±0.00^a
MAP-2 (40%CO_2, 30% N_2 & 30% O_2)	0.53±0.05^a	0.28±0.00^a	0.28±0.00^a	0.25±0.05^a	0.39±0.05^a	0.42±0.10^a
MAP-3 (50% CO_2 & 50% O_2)	0.53±0.05^a	0.32±0.05^a	0.35±0.10^a	0.32±0.05^a	0.35±0.00^a	0.39±0.05^a

In the present study, the initial FFA value Mackerel Fish sausage was 0.53 % oleic acid and then fluctuated between 0.25 and 0.42 % oleic acid (Table 43). FFA values gradually decreased up to 17th day of storage and then increasing in rest of the storage period (Table 43). There were no significant ($p>0.05$) differences in FFA values among four packaging conditions during the storage period.

FFA increased was found to be noticeably higher during the first stage of the experiment, which is in line with previous research (Rodríguez et al. 2007; Aubourg et al. 2005). This activity has been explained by a maximum lipase release from liposomes during storage, which allows closer proximity between enzyme and substrate (Sikorski & Kolakowski, 2000). However, after the initial increase, a similar decreasing trend was observed after the third month for white cheek shark in frozen storage (Nazemroaya et al. 2009). This decrease may be attributed to the depletion of

substrate and oxidation of FFA (Namulema et al. 1999). As per quality specifications for crude fish oil, maximum acceptable values of 5% FFA were proposed by Bimbo (1998). The results obtained in this study showed that the FFA content of Mackerel sausage did not reach the 5% limit during the storage period.

Table 44. Changes in TBARS (mg MDA/Kg fish) value of Mackerel Fish sausage under modified atmosphere packaging stored at 4°C temperature

Treatments	Storage period (days)					
	0	7	13	17	21	31
Not sealed pack (control)	2.05±0.06^{a}	3.12±0.19^{a}	2.92±0.29^{a}	3.37±0.28^{a}	2.86±0.07^{a}	3.35±0.11^{a}
MAP-1 (50% CO_2 & 50% N_2)	2.05±0.06^{a}	3.16±0.04^{a}	3.78±0.32^{a}	3.68±0.99^{a}	3.49±0.34^{a}	2.86±0.05^{a}
MAP-2 (40%CO_2, 30% N_2 & 30% O_2)	2.05±0.06^{a}	3.35±0.01^{a}	3.92±0.11^{a}	3.84±1.33^{a}	3.06±1.13^{a}	3.76±0.41^{a}
MAP-3 (50% CO_2 & 50% O_2)	2.05±0.06^{a}	3.21±0.21^{a}	3.97±0.91^{a}	2.92±0.01^{a}	3.52±0.13^{a}	3.73±0.11^{a}

The initial TBARS value was 2.05 mg MDA/kg Mackerel Fish sausage. The TBARS values gradually increased up to 13th day and then kept almost steady in rest of the period in all packaging conditions (Table 44). There were no such significant ($p>0.05$) differences in TBARS values among four packaging conditions during the storage period. The absence of O_2 may have caused the oxidation of PUFAs to be delayed. This finding has been agreed by Masniyom et al. (2013) and Alice et al. (2020), who found that in the absence of O_2 vacuum and MA packaged tilapia and goonch fish had the lower MDA compared to the control sample. Nunes et al. (1992) reported that the acceptability limits of TBA value for fish stored in ice were 5–8 mg MA/kg flesh. The TBARS values of all packaging conditions were within the acceptable limit.

Table 45. Changes in aerobic plate count (log CFU/g) of Mackerel Fish sausage under modified atmosphere packaging stored at 4°C temperature

Treatments	Storage period (days)					
	0	7	13	17	21	31
Not sealed pack (control)	4.26±0.11^{a}	5.11±0.03^{a}	4.48±0.12^{a}	5.56±0.11^{a}	7.04±0.03^{b}	8.32±0.02^{a}
MAP-1 (50% CO_2 & 50% N_2)	4.26±0.11^{a}	5.00±0.18^{a}	5.04±0.23^{a}	4.54±0.04^{a}	5.72±0.08^{a}	8.23±0.01^{a}
MAP-2 (40%CO_2, 30% N_2 & 30% O_2)	4.26±0.11^{a}	5.16±0.10^{a}	4.76±0.17^{a}	3.99±0.40^{a}	5.87±0.12^{a}	8.31±0.06^{a}
MAP-3 (50% CO_2 & 50% O_2)	4.26±0.11^{a}	5.14±0.13^{a}	4.56±0.11^{a}	4.99±0.67^{a}	5.90±0.11^{a}	8.16±0.12^{a}

The aerobic plate count (APC) of Mackerel Fish sausage was 4.26 log CFU/g on plate count agar medium. The APC values of Mackerel Fish sausage gradually increased with some fluctuation in all packaging conditions (Table 45). There were no significant ($p>0.05$) differences in APC observed among all packaging conditions until the 17th day of storage. However, significantly ($p<0.05$) lower APC was observed on the 21st day in all samples compared to control sample (Table 45). The APCs exceeded the 7 log CFU/g, which is the acceptable limit (ICMSF, 1986) on approximately 21st day for control, 26th day for MAP-1, MAP-2 and MAP-3 sample (Fig 48).

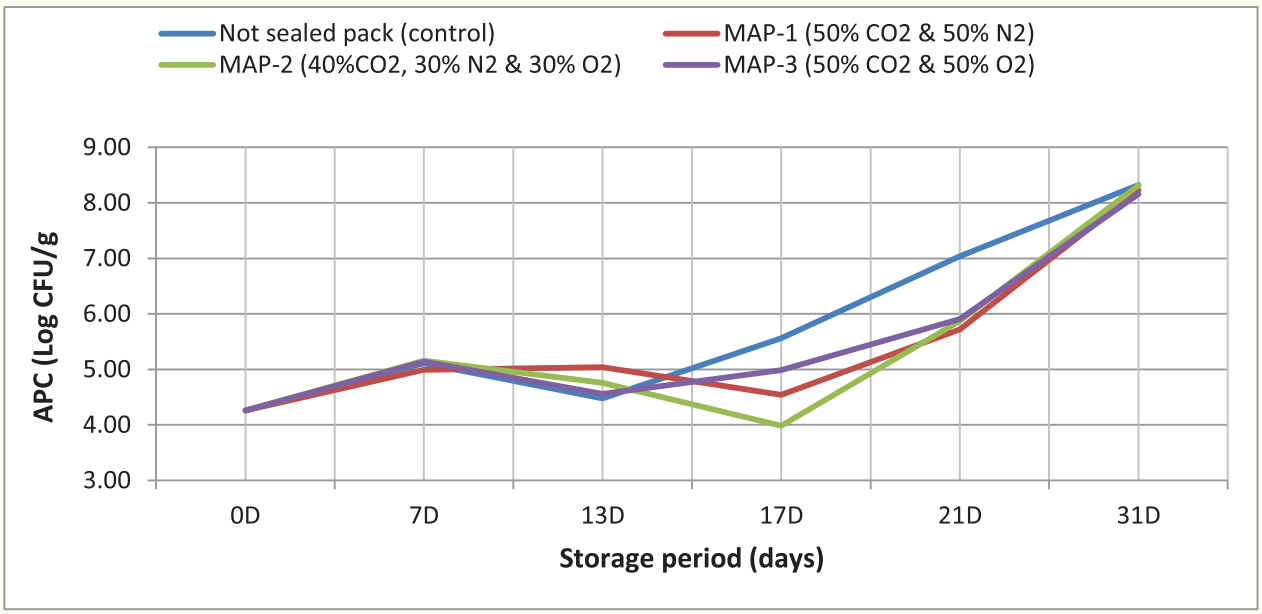


Fig 48. Changes in aerobic plate count (log CFU/g) of Mackerel Fish sausage under modified atmosphere packaging stored at 4°C temperature

11.2.3.5 Battered & breaded Tilapia fish

Table 46. Changes in pH value of Battered & breaded Tilapia under modified atmosphere packaging stored at 4°C temperature

Treatments	Storage period (days)					
	0	4	8	12	16	20
Not sealed pack (control)	5.84±0.05^{a}	6.13±0.05^{a}	5.98±0.23^{a}	6.11±0.11^{a}	6.44±0.18^{a}	
MAP-1 (50% CO_2 & 50% N_2)	5.84±0.05^{a}	6.06±0.11^{a}	6.10±0.15^{a}	6.40±0.02^{a}	5.81±0.38^{a}	5.65±0.16
MAP-2 (40%CO_2, 30% N_2 & 30% O_2)	5.84±0.05^{a}	5.95±0.19^{a}	6.21±0.01^{a}	6.23±0.364^{a}	6.25±0.18^{a}	5.36±0.19
MAP-3 (50% CO_2 & 50% O_2)	5.84±0.05^{a}	6.04±0.19^{a}	6.09±0.16^{a}	6.29±0.06^{a}	5.90±0.40^{a}	5.87±0.01

The initial pH value of the Battered & breaded Tilapia fish was 5.84. Then it increased until 12th days for MAP-1 & MAP-3 and 16th days for control & MAP-2 sample (Table 46). After that it gradually decreased in all packaging conditions. However, there were no significant ($p>0.05$) differences in pH values among all packaging conditions during the storage period. The appropriate post mortem pH level in fresh fish is typically 6.8~7.0 (Metin et al. 2001). The pH values of all samples were within the acceptable limit. The pH value dropped in MAP with CO_2 sample at later stage possibly occurred as a result of the dissolution of CO_2 in muscle tissues (Ježek and Buchtová 2012). Moreover, the fish muscle surface absorbs CO_2, thus acidifying it with carbonic acid formation (Banks et al. 1980).

Table 47. Changes in TVBN value (mg/100g) of Battered & breaded Tilapia under modified atmosphere packaging stored at 4°C temperature

Treatments	Storage period (days)					
	0	4	8	12	16	20
Not sealed pack (control)	1.82±0.20^{a}	2.38±0.20^{a}	3.36±0.40^{b}	3.72±0.11^{b}	3.43±0.13^{b}	
MAP-1 (50% CO_2 & 50% N_2)	1.82±0.20^{a}	1.40±0.40^{a}	1.54±0.20^{a}	1.82±0.20^{ab}	2.94±0.59^{ab}	2.38±0.20
MAP-2 (40%CO_2, 30% N_2 & 30% O_2)	1.82±0.20^{a}	2.10±0.20^{a}	1.63±0.33^{a}	1.26±0.20^{a}	1.82±0.20^{a}	2.10±0.20
MAP-3 (50% CO_2 & 50% O_2)	1.82±0.20^{a}	1.54±0.20^{a}	1.82±0.20^{a}	1.96±0.40^{ab}	2.14±0.25^{ab}	2.94±0.20

The TVB-N value of 30-35 mg N/100 g is generally considered a standard limit for chilled stored fish (Connell 1995). In the present study, the initial TVB-N value of Battered & breaded Tilapia was 1.82 mg/100g. The values almost gradually decreased until 8th days for control, MAP-1 & MAP-2 and 12th days for MAP-3 samples and then showed an increasing trend with some fluctuation under all packaging conditions (Table 47). Comparatively lower TVBN values were observed in all samples compared to control sample. However, there were no significant ($p>0.05$) differences in TVBN values among three packaging conditions during the storage period. The gradual increase in TVB-N values at later stage during the storage time possibly occurred due to increased bacterial growth and presence of endogenous enzymes (Ruiz-Capillas and Moral 2001, Islami et al. 2015). This finding is similar to the earlier studies, where a correlation was detected between quality deterioration and grown TVB-N values of MA packaged seafood (Özogul et al. 2004, Nayma et al. 2020).

Table 48. Changes in TBARS (mg MDA/Kg fish) value of Battered & breaded Tilapia under modified atmosphere packaging stored at 4°C temperature

Treatments	Storage period (days)					
	0	4	8	12	16	20
Not sealed pack (control)	1.29±0.06^{a}	0.52±0.24^{a}	0.41±0.29^{a}	0.45±0. 03^{c}	2.22±0.02^{d}	
MAP-1 (50% CO_2 & 50% N_2)	1.29±0.06^{a}	0.59±0.03^{a}	0.13±0.01^{a}	0.19±0.02^{a}	0.32±0.02^{a}	0.37±0.01^{a}
MAP-2 (40%CO_2, 30% N_2 & 30% O_2)	1.29±0.06^{a}	0.99±0.07^{b}	0.14±0.01^{a}	0.35±0.014^{b}	0.49±0.02^{b}	0.98±0.08^{a}
MAP-3 (50% CO_2 & 50% O_2)	1.29±0.06^{a}	0.87±0.13^{ab}	0.35±0.2^{a}	0.38±0.01^{b}	0.99±0.00^{c}	0.34±0.00^{a}

The initial value of TBARS was 1.29 mg MDA/kg Battered & breaded Tilapia. The TBARS gradually decreased with time up to 8th day and then gradually increased in rest of the storage period under all packaging conditions (Table 48). However, significantly (p <0.05) lower TBARS were observed in MAP-1 samples on the 4^{th,} 12th and 16th day of storage compared to other samples during the storage period. The absence of O_2 may have caused the oxidation of PUFAs to be delayed. This finding has been agreed by Masniyom et al. (2013) and Alice et al. (2020), who found that in the absence of O_2 vacuum and MA packaged Tilapia and goonch fish had the lower MDA compared to the control sample. The standard limit for TBARS was set as 2 mg MDA/kg fish, and beyond this limit, an unpleasant odor and taste can be formed (Connell 1995). The TBARS values did not exceed the acceptable limit (2 mg MDA/kg) during the storage period for all samples (Table 48).

Table 49. Changes in aerobic plate count (log CFU/g) of Battered & breaded Tilapia under modified atmosphere packaging stored at 4°C temperature

Treatments	Storage period (days)					
	0	4	8	12	16	20
Not sealed pack (control)	4.95±0.02^{a}	5.03±0.33^{a}	6.73±0.03^{a}	7.19±0.02^{b}	8.27±0.03^{b}	
MAP-1 (50% CO_2 & 50% N_2)	4.95±0.02^{a}	4.90±0.24^{a}	6.15±0.21^{a}	6.74±0.11^{ab}	7.36±0.06^{ab}	8.11±0.22
MAP-2 (40%CO_2, 30% N_2 & 30% O_2)	4.95±0.02^{a}	4.35±0.07^{a}	6.33±0.24^{a}	6.76±0.32^{ab}	7.01±0.36^{a}	7.93±0.18
MAP-3 (50% CO_2 & 50% O_2)	4.95±0.02^{a}	4.36±0.08^{a}	6.20±0.05^{a}	6.35±0.21^{a}	7.25±0.42^{ab}	7.59±0.01

The aerobic plate count (APC) of *B*attered & breaded Tilapia fish was 4.95 log CFU/g on plate count agar medium. The APC values of gradually increased with time in all packaging conditions (Table 49). Significantly (p<0.05) lower APC values were observed on 12th and 16th days under all packaging conditions compared to that of control sample during the storage period. The APCs

exceeded the 7 log CFU/g (ICMSF, 1986), which is the acceptable limit on approximately 10th day for control, 14th day for MAP-1, 16th day for MAP-2 and 15th day for MAP-3 sample (Fig 49).

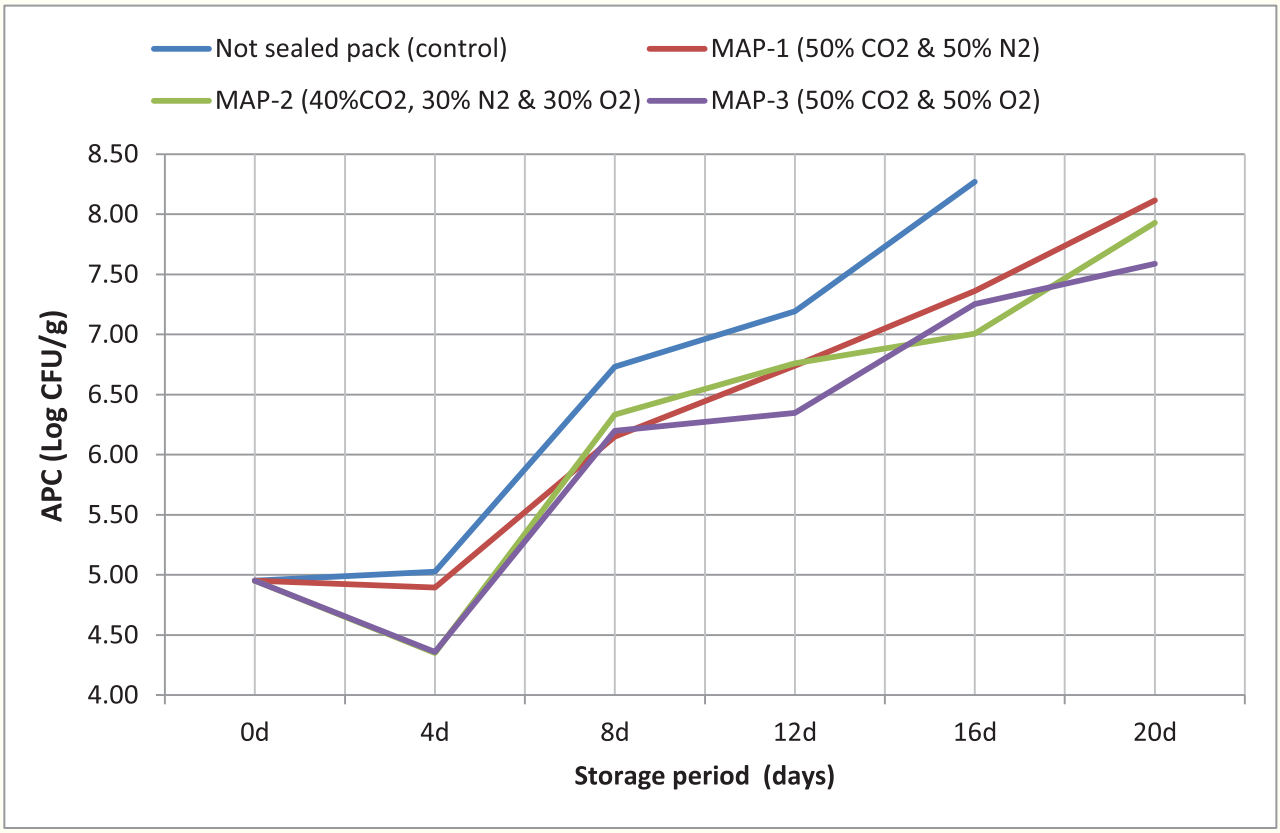


Fig 49. Changes in aerobic plate count (log CFU/g) of Battered & breaded Tilapia under modified atmosphere packaging stored at 4°C temperature

11.2.3.6 Battered & breaded white Snapper

Table 50. Changes in pH value of Battered & breaded white Snapper under modified atmosphere packaging stored at 4°C temperature

Treatments	Storage period (days)					
	0	4	8	12	16	20
Not sealed pack (control)	6.45 ± 0.04^{a}	6.46 ± 0.04^{b}	6.14 ± 0.26^{a}	6.10 ± 0.11^{c}	6.20 ± 0.04^{b}	
MAP-1 (50% CO_2 & 50% N_2)	6.45 ± 0.04^{a}	6.48 ± 0.02^{b}	6.29 ± 0.04^{a}	5.84 ± 0.12^{bc}	5.98 ± 0.08^{ab}	
MAP-2 (40%CO_2, 30% N_2 & 30% O_2)	6.45 ± 0.04^{a}	6.22 ± 0.05^{a}	6.33 ± 0.23^{a}	5.45 ± 0.01^{a}	5.87 ± 0.12^{a}	
MAP-3 (50% CO_2 & 50% O_2)	6.45 ± 0.04^{a}	6.22 ± 0.05^{a}	6.35 ± 0.14^{a}	5.70 ± 0.08^{ab}	5.77 ± 0.02^{a}	

The initial pH of the present study of Battered & breaded white Snapper was 6.45 and then gradually decreased with some fluctuation during the entire period of time (Table 50). However,

significantly ($p<0.05$) lower pH values were observed on 12th and 16th day of storage in all packaging conditions compared to that of control sample. The appropriate post mortem pH level in fresh fish is typically 6.8~7.0 (Metin et al. 2001). The pH values of all samples were within the acceptable limit. The dropped in pH value in MAP with CO_2 sample at later stage possibly occurred as a result of the dissolution of CO_2 in muscle tissues (Ježek and Buchtová 2012). Moreover, the fish muscle surface absorbs CO_2, thus acidifying it with carbonic acid formation (Banks et al. 1980).

Table 51. Changes in TVBN value (mg/100g) of Battered & breaded white Snapper fish under modified atmosphere packaging stored at 4°C temperature

Treatments	Storage period (days)					
	0	4	8	12	16	20
Not sealed pack (control)	4.06±1.39^{a}	6.08±0.28^{a}	6.16±0.79^{b}	5.60±0.40^{a}	5.71±0.24^{b}	
MAP-1 (50% CO_2 & 50% N_2)	4.06±1.39^{a}	4.90±0.99^{a}	3.08±0.40^{a}	2.94±0.20^{a}	3.10±0.03^{a}	
MAP-2 (40%CO_2, 30% N_2 & 30% O_2)	4.06±1.39^{a}	5.18±0.99^{a}	3.22±0.20^{a}	2.38±1.39^{a}	2.88±0.68^{a}	
MAP-3 (50% CO_2 & 50% O_2)	4.06±1.39^{a}	5.74±0.59^{a}	4.48±0.00^{ab}	4.62±0.99^{a}	5.12±0.28^{b}	

The TVB-N value of 30-35 mg N/100 g is generally considered a standard limit for chilled stored fish (Connell 1995). The initial TVB-N value was 4.06 mg/100 g in Battered & breaded white Snapper sample (Table 51), and then increased until 4th day of all samples. After that TVBN values gradually decreased in rest of the period. Comparatively lower TVB-N value was found in three samples compared to control pack. Significant ($p<0.05$) lower TVBN values were observed in MAP-1 & 2 samples compared to that of control & MAP-3 sample on 8th and 16th day of storage. However, TVBN values were within the acceptable limit in all samples during entire storage period.

Table 52. Changes in FFA (% oleic acid) value of Battered & breaded white Snapper under modified atmosphere packaging stored at 4°C temperature

Treatments	Storage period (days)					
	0	4	8	12	16	20
Not sealed pack (control)	0.35±0.10^{a}	0.39±0.05^{a}	0.35±0.20^{a}	0.42±0.10^{a}	0.46±0.05^{a}	
MAP-1 (50% CO_2 & 50% N_2)	0.35±0.10^{a}	0.32±0.05^{a}	0.32±0.05^{a}	0.46±0.05^{a}	0.46±0.05^{a}	
MAP-2 (40%CO_2, 30% N_2 & 30% O_2)	0.35±0.10^{a}	0.28±0.00^{a}	0.32±0.05^{a}	0.39±0.05^{a}	0.42±0.10^{a}	
MAP-3 (50% CO_2 & 50% O_2)	0.35±0.10^{a}	0.32±0.05^{a}	0.32±0.05^{a}	0.35±0.00^{a}	0.39±0.05^{a}	

In the present study, the initial FFA value of Battered & breaded white Snapper was 0.35 % oleic acid and then fluctuated between 0.25 and 0.42 % oleic acid (Table 52). FFA values gradually

increased in all packaging conditions with time during entire storage period. There were no significant ($p>0.05$) differences in FFA values among four packaging conditions during the storage period. As per quality specifications for crude fish oil, maximum acceptable values of 5% FFA were proposed by Bimbo (1998). The results obtained in this study showed that the FFA content of *B*attered & breaded white Snapper did not reach the 5% limit during the storage period.

Table 53. Changes in TBARS (mg MDA/Kg fish) value of Battered & breaded white Snapper under modified atmosphere packaging stored at 4°C temperature

Treatments	Storage period (days)					
	0	4	8	12	16	20
Not sealed pack (control)	1.51 ± 0.27^{a}	1.93 ± 0.16^{a}	1.14 ± 0.14^{c}	2.15 ± 0.32^{b}	2.49 ± 0.26^{c}	
MAP-1 (50% CO_2 & 50% N_2)	1.51 ± 0.27^{a}	1.53 ± 0.23^{a}	0.35 ± 0.01^{a}	0.74 ± 0.58^{a}	1.14 ± 0.01^{a}	
MAP-2 (40%CO_2, 30% N_2 & 30% O_2)	1.51 ± 0.27^{a}	1.82 ± 0.34^{a}	0.94 ± 0.06^{b}	0.88 ± 0.45^{a}	1.55 ± 0.15^{ab}	
MAP-3 (50% CO_2 & 50% O_2)	1.51 ± 0.27^{a}	1.97 ± 0.05^{a}	0.43 ± 0.02^{a}	0.88 ± 0.04^{a}	1.88 ± 0.04^{b}	

The initial value of TBARS was 1.51 mg MDA/kg Battered & breaded white Snapper. The TBARS gradually decreased with time up to 8th day and then gradually increased in rest of the storage period under all packaging conditions (Table 53). However, significantly (p <0.05) lower TBARS were observed in MAP-1 samples on the 8^{th,} 12th and 16th day of storage compared to control sample and some cases other samples during the storage period. The absence of O_2 may have caused the oxidation of PUFAs to be delayed. This finding has been agreed by Masniyom et al. (2013) and Alice et al. (2020), who found that in the absence of O_2 vacuum and MA packaged tilapia and goonch fish had the lower MDA compared to the control sample. The standard limit for TBARS was set as 2 mg MDA/kg fish, and beyond this limit, an unpleasant odor and taste can be formed (Connell 1995). The TBARS values did not exceed the acceptable limit (2 mg MDA/kg) during the storage period for all samples except control sample where it exceeded on 12th day of storage (Table 53).

Table 54. Changes in aerobic plate count (log CFU/g) of Battered & breaded white Snapper under modified atmosphere packaging stored at 4°C temperature

Treatments	Storage period (days)					
	0	4	8	12	16	20
Not sealed pack (control)	4.58 ± 0.05^{a}	5.19 ± 0.08^{a}	6.25 ± 0.19^{a}	7.62 ± 0.16^{b}	8.32 ± 0.13^{b}	
MAP-1 (50% CO_2 & 50% N_2)	4.58 ± 0.05^{a}	5.24 ± 0.11^{a}	6.35 ± 0.39^{a}	6.96 ± 0.06^{a}	7.96 ± 0.05^{b}	8.61 ± 0.23
MAP-2 (40%CO_2, 30% N_2 & 30% O_2)	4.58 ± 0.05^{a}	5.19 ± 0.04^{a}	5.96 ± 0.09^{a}	6.63 ± 0.12^{a}	6.88 ± 0.09^{a}	7.96 ± 0.24
MAP-3 (50% CO_2 & 50% O_2)	4.58 ± 0.05^{a}	5.13 ± 0.14^{a}	6.39 ± 0.00^{a}	7.03 ± 0.08^{a}	8.03 ± 0.08^{b}	8.55 ± 0.13

The aerobic plate count (APC) of *B*attered & breaded white Snapper was 4.58 log CFU/g on plate count agar medium. The APC values of battered & breaded white Snapper gradually increased with progress to time in all packaging conditions (Table 54). There were no significant ($p>0.05$) differences in APC values up to 8th day in all packaging conditions during the storage period. However, significantly ($p<0.05$) lower APC values were observed on 12th and 16th days in MAP-2 sample compared to that of control sample during the storage period. The APCs exceeded the 7 log CFU/g (ICMSF, 1986), which is the acceptable limit on approximately 10th day for control, 12th day for MAP-1, 16th day for MAP-2 and 12th day for MAP-3 sample (Fig 50).

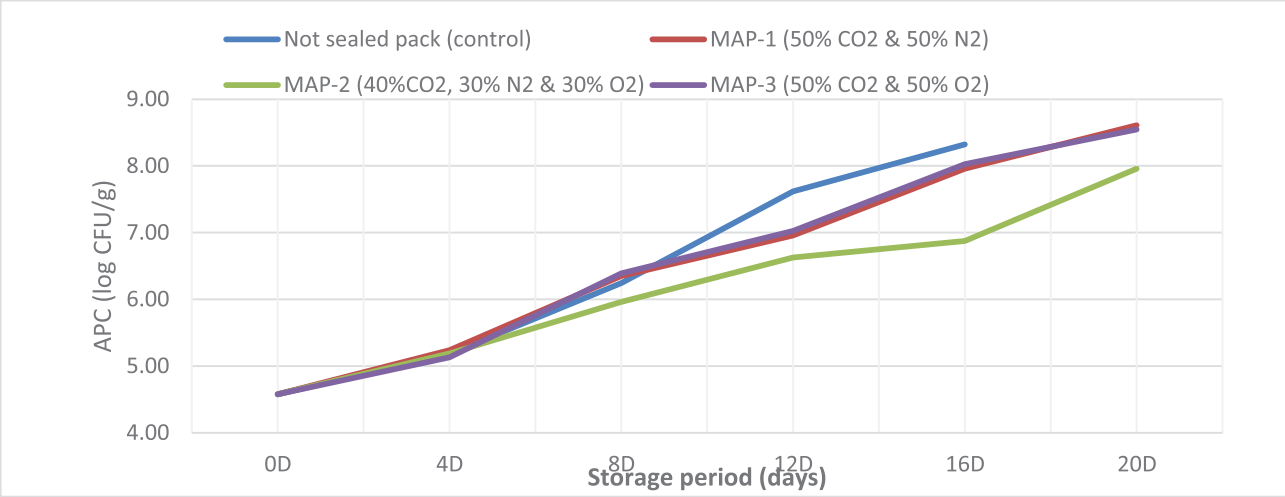


Fig 50. Changes in aerobic plate count (log CFU/g) of Battered & breaded white Snapper under modified atmosphere packaging stored at 4°C temperature

11.2.3.7 Sardine Fish marinades

Table 55. Changes in pH value of Sardine Fish marinades under modified atmosphere packaging stored at 4°C temperature

Treatments	Storage period (days)				
	0	7	14	24	34
Not sealed pack (control)	3.67±0.04^{a}	3.88±0.09^{a}	4.42±0.08^{b}	4.46±0.14^{b}	4.47±0.04^{c}
MAP-1 (50% CO_2 & 50% N_2)	3.67±0.04^{a}	3.82±0.01^{a}	4.04±0.01^{a}	4.00±0.01^{a}	3.72±0.01^{a}
MAP-2 (40%CO_2, 30% N_2 & 30% O_2)	3.67±0.04^{a}	3.80±0.06^{a}	4.00±0.01^{a}	3.97±0..04^{a}	3.82±0.03^{ab}
MAP-3 (50% CO_2 & 50% O_2)	3.67±0.04^{a}	3.81±0.13^{a}	4.07±0.01^{a}	4.11±0.04^{a}	3.90±0.00^{b}

The initial pH of the present study of Sardine Fish marinades was 3.67. Then, the pH values gradually increased up to 24th day and then steady in control sample and decreased in another sample (Table 55). There were no significant ($p>0.05$) differences in pH values up to 7th day among all packaging conditions during the storage period. However, significantly ($p<0.05$) lower pH was observed on 14th and 24th day in all packaging conditions compared to that of control sample. The appropriate post mortem pH level in fresh fish is typically 6.8~7.0 (Metin et al. 2001). The pH values of all samples were well below the acceptable limit as because of using acetic acid during preparation of Fish marinades. It was evident in the past study that the result of increased pH was linked with the generation of basic components such as ammonia, dimethylamine, trimethylamine, and other biogenic amines, as well as microbial spoilage (Goulas and Kontominas, 2007).

Table 56. Changes in TVBN value (mg/100g) of Sardine Fish marinades under modified atmosphere packaging stored at 4°C temperature

Treatments	Storage period (days)				
	0	7	14	24	34
Not sealed pack (control)	2.80±0.79^{a}	2.80±0.40^{a}	2.94±0.20^{a}	2.94±0.20^{a}	3.64±0.40^{a}
MAP-1 (50% CO_2 & 50% N_2)	2.80±0.79^{a}	2.38±0.20^{a}	3.64±0.00^{a}	3.36±0.40^{a}	4.20±0.00^{a}
MAP-2 (40%CO_2, 30% N_2 & 30% O_2)	2.80±0.79^{a}	2.24±0.40^{a}	3.08±0.00^{a}	2.38±0.20^{a}	3.22±0.20^{a}
MAP-3 (50% CO_2 & 50% O_2)	2.80±0.79^{a}	2.38±0.20^{a}	3.08±0.40^{a}	3.36±0.79^{a}	5.88±0.40^{b}

The initial TVB-N value was 2.80 mg/100 g in Sardine Fish marinades sample, and then a gradual increase was observed during the rest of the storage period. The highest TVB-N value was found at 5.88 mg/100 g on the 34th day for MAP-3 sample. However, there were no such significant ($p>0.05$) differences in TVBN values among almost all packaging conditions during the storage period (Table 56). The TVB-N value of 30-35 mg N/100 g is generally considered a standard limit for chilled stored fish (Connell 1995). TVBN values of all samples were within the acceptable limit during the entire storage period. The gradual increase in TVB-N values during the storage time possibly occurred due to increased bacterial growth and presence of endogenous enzymes (Ruiz-Capillas and Moral 2001, Islami et al. 2015). This finding is similar to the earlier studies, where a correlation was detected between quality deterioration and grown TVB-N values of MA packaged seafood (Özogul et al. 2004, Nayma et al. 2020).

Table 57. Changes in FFA (% oleic acid) value of Sardine Fish marinades under modified atmosphere packaging stored at 4°C temperature

Treatments	Storage period (days)				
	0	7	14	24	34
Not sealed pack (control)	0.99±0.20^{a}	0.67±0.15^{a}	0.74±0.05^{a}	0.53±0.25^{a}	0.63±0.10^{a}
MAP-1 (50% CO_2 & 50% N_2)	0.99±0.20^{a}	0.78±0.10^{a}	0.92±0.00^{a}	0.78±0.10^{a}	0.78±0.20^{a}
MAP-2 (40%CO_2, 30% N_2 & 30% O_2)	0.99±0.20^{a}	0.81±0.05^{a}	0.78±0.00^{a}	0.74±0.05^{a}	0.81±0.05^{a}
MAP-3 (50% CO_2 & 50% O_2)	0.99±0.20^{a}	0.81±0.15^{a}	0.78±0.10^{a}	0.71±0.20^{a}	0.67±0.15^{a}

In the present study, the initial FFA value Sardine Fish marinades were 0.99 % oleic acid and then fluctuated between 0.99 and 0.52 % oleic acid (Table 57). FFA values gradually decreased during the entire storage period with some fluctuations. There were no significant ($p > 0.05$) differences in FFA values among four packaging conditions during the storage period.

Initial higher FFA was found to be noticeably higher during the first stage of the experiment, which is in line with previous research (Rodríguez et al. 2007; Aubourg et al. 2005). This activity has been explained by a maximum lipase release from liposomes during storage, which allows closer proximity between enzyme and substrate (Sikorski & Kolakowski, 2000). However, after the initial increase, a similar decreasing trend was observed which may be attributed to the depletion of substrate and oxidation of FFA (Namulema et al. 1999). As per quality specifications for crude fish oil, maximum acceptable values of 5% FFA were proposed by Bimbo (1998). The results obtained in this study showed that the FFA content of Sardine marinades did not reach the 5% limit during the storage period.

Table 58. Changes in TBARS (mg MDA/Kg fish) value of Sardine Fish marinades under modified atmosphere packaging stored at 4°C temperature

Treatments	Storage period (days)				
	0	7	14	24	34
Not sealed pack (control)	2.79±0.11^{a}	9.79±0.57^{d}	5.40±4.99^{a}	4.01±0.52^{b}	3.52±0.02^{a}
MAP-1 (50% CO_2 & 50% N_2)	2.79±0.11^{a}	3.39±0.10^{a}	1.56±0.14^{a}	2.65±0.04^{a}	4.03±0.02^{b}
MAP-2 (40%CO_2, 30% N_2 & 30% O_2)	2.79±0.11^{a}	6.93±0.65^{b}	2.81±0.53^{a}	3.11±0.05^{a}	4.46±0.03^{c}
MAP-3 (50% CO_2 & 50% O_2)	2.79±0.11^{a}	8.52±0.09^{c}	3.25±0.09^{a}	4.59±0.08^{b}	5.06±0.12^{d}

The initial TBARS value was 2.79 mg MDA/kg Sardine Fish marinades. The TBARS values initially increased on 7th day of storage and then gradually decreased with some fluctuation in rest of the period in all packaging conditions (Table 58). Significantly ($p<0.05$) lower TBARS values observed in MAP-1 samples on 7th, 24th and 34th day of storage compared to that of control and MAP-3 samples. The absence of O_2 may have caused the oxidation of PUFAs to be delayed. This finding has been agreed by Masniyom et al. (2013) and Alice et al. (2020), who found that in the absence of O_2 vacuum and MA packaged Tilapia and goonch fish had the lower MDA compared to the control sample. The standard limit for TBARS was set as 2 mg MDA/kg fish, and beyond this limit, an unpleasant odor and taste can be formed (Connell 1995). The TBARS values exceeded the acceptable limit (2 mg MDA/kg) in all samples during the storage period (Table 58). According to these results, it would be difficult to set limits for TBA levels, and TBA values may not give actual rate of lipid oxidation since malonaldehyde can interact with other components of fish such as nucleosides, nucleic acid, proteins and amino acids of phospholipids (Aubourg, 1993), and produce secondary metabolites that include carbohydrates, furfural, alkenals, alkadienals and other aldehydes and ketones (Botsoglou et al., 1994).

Table 59. Changes in aerobic plate count (log CFU/g) of Sardine Fish marinades under modified atmosphere packaging stored at 4°C temperature

Treatments	Storage period (days)				
	0	7	14	24	34
Not sealed pack (control)	5.04±0.03^{a}	4.30±0.28^{a}	4.17±0.31^{a}	7.02±0.26^{b}	7.47±0.10^{b}
MAP-1 (50% CO_2 & 50% N_2)	5.04±0.03^{a}	3.82±0.06^{a}	3.91±0.18^{a}	6.18±0.03^{a}	7.35±0.12^{ab}
MAP-2 (40%CO_2, 30% N_2 & 30% O_2)	5.04±0.03^{a}	3.87±0.27^{a}	3.68±0.27^{a}	6.17±0.03^{a}	6.92±0.03^{a}
MAP-3 (50% CO_2 & 50% O_2)	5.04±0.03^{a}	3.73±0.12^{a}	3.61±0.04^{a}	6.16±0.00^{a}	7.28±0.21^{ab}

The aerobic plate count (APC) of Sardine Fish marinades was 5.04 log CFU/g on plate count agar medium. The APC values of Sardine marinades gradually decreased until 14th day in all packaging conditions and then gradually increased with some fluctuation in rest of the storage period in all packaging conditions (Table 59). The reduction rate in the initial APC of the marinated fish could be attributed to the combined effect of the acid and the salt in the marinating solution. However, in the later stage, some bacteria could adapt the environment and grow gradually. Similar reduction trend was also observed in marinated Pacific saury and pink shrimp (Cadun et al. 2008; Sallam, 2008). There were no significant ($p>0.05$) differences in APC observed among all packaging conditions until the 14th day of storage. However, significantly ($p<0.05$) lower APC was observed on the 24th and 34th day of storage in all samples compared to control sample (Table 59). The APCs exceeded the 7 log CFU/g (ICMSF, 1986), which is the acceptable limit on approximately 24th day for control, 31th day for MAP-1, 34th day for MAP-2 and 31th day for MAP-3 sample (Fig 51).

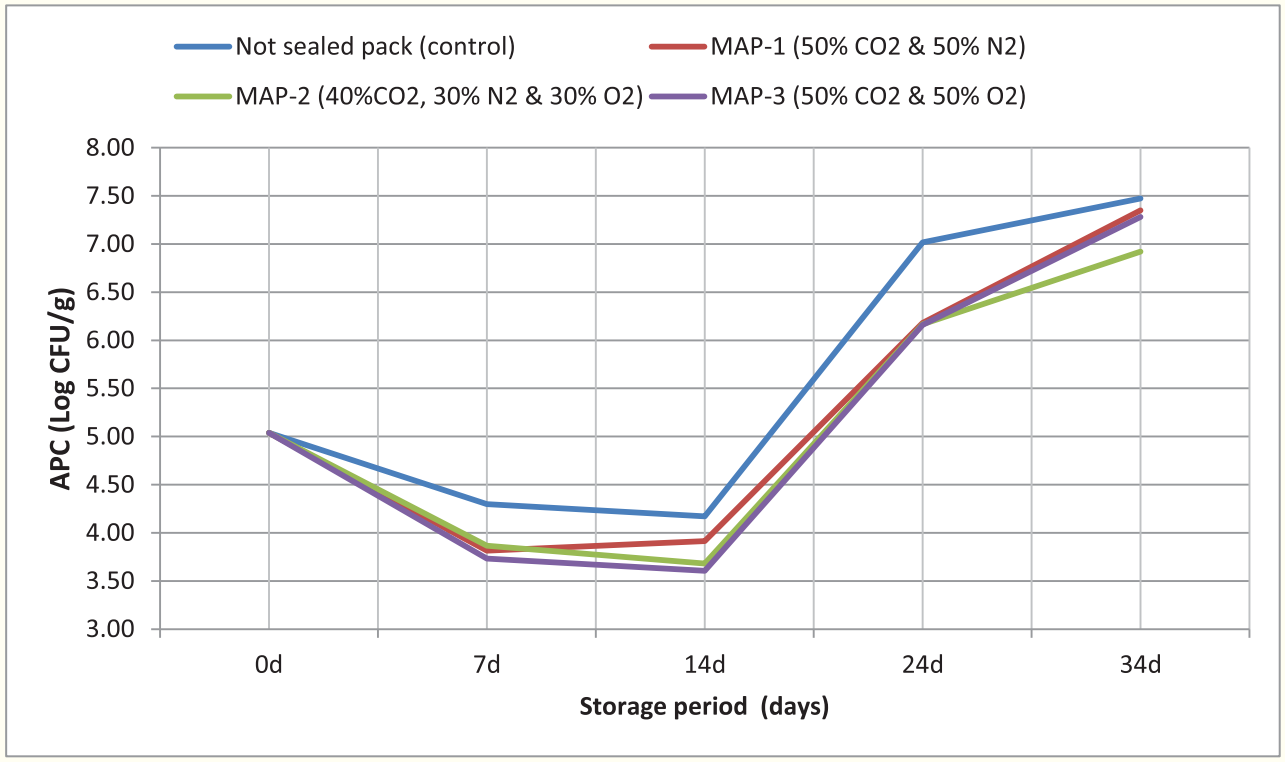


Fig 51. Changes in aerobic plate count (log CFU/g) of Sardine Fish marinades under modified atmosphere packaging stored at 4°C temperature

11.2.3.8 Tilapia Fish crackers

Table 60. Changes in pH value of Tilapia Fish crackers under modified atmosphere packaging stored at room temperature

Treatments	Storage period (Months)						
	0	**1**	**2**	**3**	**4**	**5**	**6**
Air pack (control)	8.86±0.66^{a}	9.18±0.03^{b}	8.97±0.02^{b}	8.90±0.08^{a}	9.03±0.01^{ab}	8.64±0.06^{a}	8.68±0.04^{a}
MAP-1 (100% N_2)	8.86±0.66^{a}	9.18±0.01^{b}	9.03±0.03^{b}	9.04±0.11^{a}	9.12±0.02^{b}	8.88±0.03^{b}	8.95±0.06^{b}
MAP-2 (50% N_2 & 50% CO_2)	8.86±0.66^{a}	8.51±0.12^{a}	8.66±0.01^{a}	8.81±0.11^{a}	8.95±0.03^{a}	8.75±0.01^{ab}	8.84±0.08^{ab}

The initial pH of the RTE Tilapia Fish crackers was 8.86 and then fluctuated between 8.51 to 9.18 during the storage period. The appropriate post mortem pH level in fresh fish is typically 6.8~7.0

(Metin et al. 2001). The higher pH in the fish crackers is due to use of sodium bicarbonate during preparation of fish crackers. However, significantly (p <0.05) lower pH was observed until 4th month in MAP-2 sample compared to that of other samples (Table 60). The drop in pH value in MAP-2 using 50% CO_2 sample possibly occurred as a result of the dissolution of CO_2 in muscle tissues (Ježek and Buchtová 2012). Moreover, fish muscle surface absorbs CO_2, thus acidifying it with carbonic acid formation (Banks et al. 1980).

Table 61. Changes in FFA value (% oleic acid) in Tilapia Fish crackers under modified atmosphere packaging stored at room temperature

Treatments	Storage period (Months)						
	0	1	2	3	4	5	6
Air pack (control)	0.21±0.00^a	0.25±0.05^a	0.35±0.10^a	0.25±0.05^a	0.25±0.05^a	0.28±0.00^a	0.25±0.05^a
MAP-1 (100% N_2)	0.21±0.00^a	0.32±0.05^a	0.32±0.05^a	0.25±0.05^a	0.21±0.00^a	0.32±0.05^a	0.32±0.05^a
MAP-2 (50% N_2 & 50% CO_2)	0.21±0.00^a	0.35±0.10^a	0.32±0.05^a	0.25±0.05^a	0.25±0.05^a	0.32±0.05^a	0.39±0.05^a

In the present study, the initial FFA value Tilapia Fish crackers were 0.21 % oleic acid and then fluctuated between 0.21 and 0.39 % oleic acid (Table 61). Initially, the FFA values gradually increased up to 2nd month and then decreased with increasing at the end of storage period. However, there were no significant (p>0.05) differences in FFA values among three packaging conditions during the storage period.

FFA is considered to have adverse effects on protein properties; thus, FFA accumulation has been associated with a lack of acceptability and oxidizes faster than higher molecular weight lipid groups (Sikorski & Kołakowska, 1994). Rise of FFA was found to be noticeably higher during the first stage of the experiment (months 0–2), which is in line with previous research (Rodríguez et al. 2007; Aubourg et al. 2005). This activity has been explained by a maximum lipase release from liposomes during storage, which allows closer proximity between enzyme and substrate (Sikorski & Kolakowski, 2000). However, after the initial increase, a similar decreasing trend was observed after the third month for frozen white cheek shark in frozen storage (Nazemroaya et al. 2009). This decrease may be attributed to the depletion of substrate and oxidation of FFA (Namulema et al. 1999). As per quality specification for crude fish oil, the maximum acceptable value of 5% FFA were proposed (Bimbo, 1998). The results obtained in this study showed that the FFA content of Tilapia crackers did not exceed the 5% limit during the storage period.

Table 62. Changes in TBARS (mg MDA/Kg fish) value of Tilapia Fish crackers under modified atmosphere packaging stored at room temperature

Treatments	Storage period (Months)						
	0	1	2	3	4	5	6
Air pack (control)	3.83±0.49^{a}	2.46±0.05^{a}	3.72±0.42^{a}	3.70±0.35^{a}	2.88±0.71^{a}	3.63±0.80^{a}	4.32±0.16^{a}
MAP-1 (100% N_2)	3.83±0.49^{a}	2.81±0.13^{a}	4.77±0.64^{a}	4.27±0.22^{a}	3.41±0.63^{a}	3.41±0.13^{a}	5.23±0.16^{b}
MAP-2 (50% N_2 & 50% CO_2)	3.83±0.49^{a}	2.28±0.48^{a}	4.38±0.72^{a}	3.95±0.55^{a}	3.22±0.44^{a}	3.58±0.67^{a}	5.05±0.25^{ab}

The initial value of TBARS was 3.83 mg MDA/kg Tilapia Fish crackers and then decreased on the 1st month. Then the values increased on the 2nd month and then fluctuated in rest of the period (Table 62). The increase in TBARS indicated formation of secondary lipid oxidation products, while the decrease in TBA values may represent the breakdown of the malonaldehyde because of tertiary degradation (Pezeshk et al. 2011). There were no such significant ($p>0.05$) differences in TBARS values among three packaging conditions during the storage period. Nunes et al. (1992) reported that the acceptability limits of TBA value for fish stored in ice were 5–8 mg MA/kg flesh. The TBARS values of all packaging conditions were within the acceptable limit.

Table 63. Changes in aerobic plate count (log CFU/g) of Tilapia Fish crackers under modified atmosphere packaging stored at room temperature

Treatments	Storage period (Months)						
	0	1	2	3	4	5	6
Air pack (control)	**4.30**±0.23^{a}	**4.96**±0.74^{a}	**4.71**±0.57^{a}	**5.33**±0.30^{a}	**5.57**±0.28^{b}	**6.82**±0.56^{b}	**7.56**±0.09^{b}
MAP-1 (100% N_2)	**4.30**±0.23^{a}	**5.16**±0.51^{a}	**5.70**±0.12^{a}	**5.61**±0.22^{a}	**5.66**±0.09^{a}	**5.20**±0.24^{a}	**7.22**±0.03^{a}
MAP-2 (50% N_2 & 50% CO_2)	**4.30**±0.23^{a}	**4.72**±0.47^{a}	**5.32**±0.09^{a}	**5.34**±0.16^{a}	**5.15**±0.15^{a}	**5.15**±0.19^{a}	**7.03**±0.09^{a}

The aerobic plate count (APC) of RTE Tilapia Fish crackers was 4.30 log CFU/g on plate count agar medium. The APC values of Tilapia crackers gradually increased in all packaging conditions with storage period (Table 63). There was no significant difference ($p>0.05$) was observed in APCs among all treated samples until the 3rd month of storage. However, significantly ($p<0.05$) lower APC values were observed on 4th, 5th and 6th month under all packaging conditions compared to that of control sample during the storage period. According to ICMSF (1986), the upper acceptable limit of aerobic plate counts (APC) for Ready-to-eat fishery products is 6 log CFU/g.

In the current study, the APCs exceeded the 6 log CFU/g on approximately 4th month for air pack, 5th month for, MAP-1 & MAP-2 sample (Fig 52).

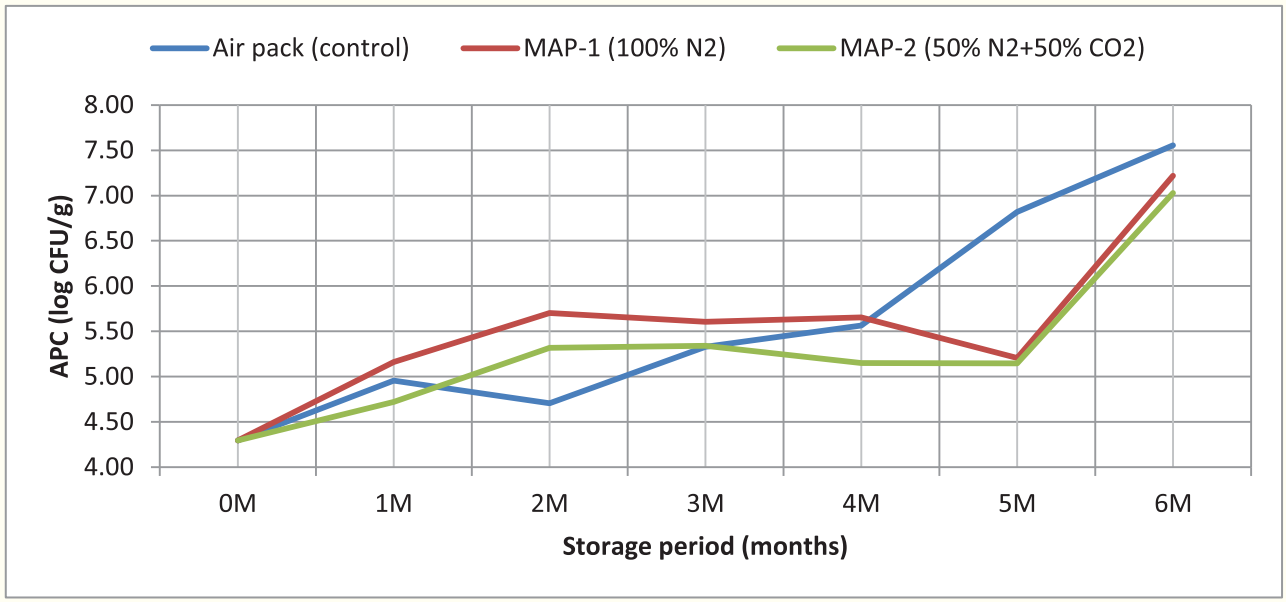


Fig 52. Changes in aerobic plate count (log CFU/g) of Tilapia Fish crackers under modified atmosphere packaging stored at room temperature

11.2.3.9 Tuna Fish crackers

Table 64. Changes in pH value of Tuna Fish crackers under modified atmosphere packaging stored at room temperature

Treatments	Storage period (Months)						
	0	**1**	**2**	**3**	**4**	**5**	**6**
Air pack (control)	7.29±0.05^{a}	7.11±0.05^{a}	7.05±0.03^{a}	6.96±0.03^{a}	6.84±0.05^{a}	7.08±0.00^{a}	6.96±0.06^{a}
MAP-1 (100% N_2)	7.29±0.05^{a}	7.19±0.00^{a}	7.17±0.03^{a}	7.13±0.01^{b}	7.07±0.01^{b}	7.27±0.03^{b}	7.00±0.01^{a}
MAP-2 (50% N_2 & 50% CO_2)	7.29±0.05^{a}	7.16±0.01^{a}	7.11±0.06^{a}	7.08±0.01^{b}	7.01±0.00^{b}	7.17±0.07^{ab}	6.95±0.00^{a}

The initial pH of RTE Tuna Fish crackers was 7.29 and then fluctuated between 6.84 and 7.29 during the storage period. The appropriate post mortem pH level in fresh fish is typically 6.8~7.0 (Metin et al. 2001). The higher pH in the Fish crackers is due to use of sodium bicarbonate during preparation of Fish crackers. In this study, the pH values gradually decreased up to 4th month during storage period. There were no significant ($p>0.05$) differences in pH values among three packaging conditions up to 2nd month of the storage period. However, significantly ($p<0.05$) higher pH was observed in MAP samples on 3rd, 4th and 5th month of storage compared to that of control sample. (Table 64).

Table 65. Changes in FFA value (% oleic acid) of Tuna Fish crackers under modified atmosphere packaging stored at room temperature

Treatments	Storage period (Months)						
	0	1	2	3	4	5	6
Air pack (control)	0.18±0.05^a	0.21±0.00^a	0.28±0.00^a	0.21±0.00^a	0.25±0.05^a	0.35±0.10^a	0.42±0.20^a
MAP-1 (100% N_2)	0.18±0.05^a	0.28±0.00^a	0.32±0.05^a	0.28±0.00^a	0.28±0.00^a	0.35±0.00^a	0.28±0.00^a
MAP-2 (50% N_2 & 50% CO_2)	0.18±0.05^a	0.25±0.05^a	0.39±0.05^a	0.18±0.05^a	0.25±0.05^a	0.35±0.10^a	0.39±0.05^a

In the present study, the initial FFA value Tuna Fish crackers were 0.18 % oleic acid and then fluctuated between 0.21 and 0.42 % oleic acid (Table 65). The FFA values initially gradually increased up to 2nd month and then decreased with increasing at the end of storage period. However, there were no significant ($p>0.05$) differences in FFA values among three packaging conditions during the storage period. FFA increased was found to be noticeably higher during the first stage of the experiment (months 0–2), which is in line with previous research (Rodríguez et al. 2007; Aubourg et al. 2005). This activity has been explained by a maximum lipase release from liposomes during storage, which allows closer proximity between enzyme and substrate (Sikorski & Kolakowski, 2000). However, after the initial increase, a similar decreasing trend was observed after the third month for white cheek shark in frozen storage (Nazemroaya et al. 2009). This decrease may be attributed to the depletion of substrate and oxidation of FFA (Namulema et al. 1999). As per quality specifications for crude fish oil, maximum acceptable values of 5% FFA were proposed by Bimbo (1998). The results obtained in this study showed that the FFA content of Tuna crackers did not reach the 5% limit during the storage period.

Table 66. Changes in TBARS (MDA/Kg fish) value of Tuna Fish crackers under modified atmosphere packaging stored at room temperature

Treatments	Storage period (Months)						
	0	1	2	3	4	5	6
Air pack (control)	3.37±0.35^a	3.30±0.34^a	2.99±0.04^a	3.32±0.71^a	3.18±0.06^a	3.23±0.73^a	4.01±0.62^a
MAP-1 (100% N_2)	3.37±0.35^a	3.54±0.38^a	3.14±0.20^a	3.03±0.10^a	3.73±0.86^a	3.66±0.29^a	4.35±0.02^a
MAP-2 (50% N_2 & 50% CO_2)	3.37±0.35^a	3.33±0.31^a	3.17±0.01^a	3.05±0.39^a	3.40±0.04^a	3.58±0.06^a	4.18±0.09^a

The initial value of TBARS was 3.37 mg MDA/kg Tuna Fish crackers. The TBARS values were almost steady until 4th month and gradually increased in rest of the storage period in all packaging conditions (Table 66). There were no significant ($p>0.05$) differences in TNBARS values among three packaging conditions during the storage period. Nunes et al. (1992) reported that the

acceptability limits of TBA value for fish stored in ice were 5–8 mg MA/kg flesh. The TBARS values of all packaging conditions were within the acceptable limit.

Table 67. Changes in aerobic plate count (log CFU/g) of Tuna Fish crackers under modified atmosphere packaging stored at room temperature

Treatments	Storage period (Months)						
	0	**1**	**2**	**3**	**4**	**5**	**6**
Air pack (control)	5.01 ± 0.48^{a}	5.66 ± 0.73^{a}	5.08 ± 0.79^{a}	5.85 ± 0.69^{a}	5.61 ± 0.38^{a}	6.61 ± 0.15^{b}	6.66 ± 0.13^{b}
MAP-1 (100% N_2)	5.01 ± 0.48^{a}	5.14 ± 0.85^{a}	5.23 ± 0.31^{a}	5.29 ± 0.69^{a}	5.12 ± 0.31^{a}	5.70 ± 0.07^{ab}	5.68 ± 0.03^{a}
MAP-2 (50% N_2 & 50% CO_2)	5.01 ± 0.48^{a}	4.43 ± 0.45^{a}	4.69 ± 0.12^{a}	4.74 ± 0.03^{a}	4.81 ± 0.11^{a}	5.18 ± 0.38^{a}	5.71 ± 0.17^{a}

The aerobic plate count (APC) of RTE Tuna Fish crackers was 5.01 log CFU/g on plate count agar medium. The APC values of Tuna Fish Crackers gradually increased with time in all packaging conditions (Table 67). There was no significant difference (p >0.05) was observed in APCs among all treated samples until the 4th month of storage. However, significantly ($p<0.05$) lower APC values were observed on 5th and 6th month under all packaging conditions compared to that of control sample during the storage period. According to ICMSF (1986), the upper acceptable limit of aerobic plate counts (APC) for Ready-to-eat fishery products is 6 log CFU/g. In the current study, the APCs exceeded the 6 log CFU/g on approximately 4th month for air pack, 6th month for MAP-1 & MAP-2 sample (Fig 53).

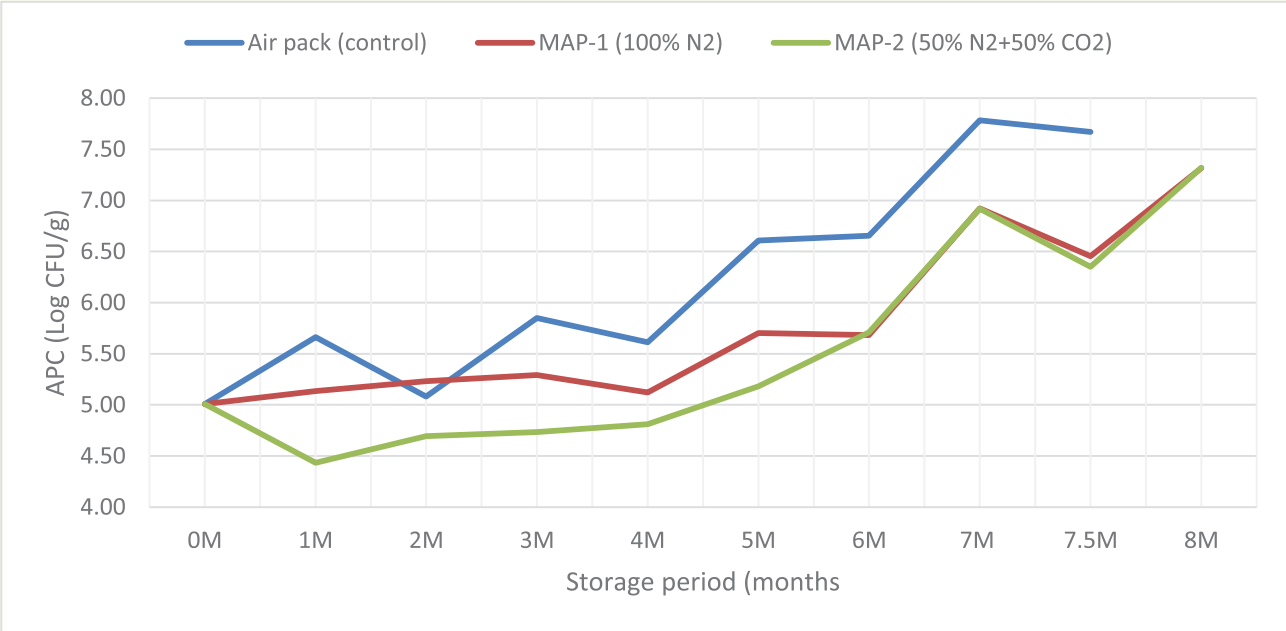


Fig 53. Changes in aerobic plate count (log CFU/g) of Tuna Fish crackers under modified atmosphere packaging stored at room temperature

11.2.4. Consumer's acceptability of RTC and RTE fishery products in the market

Consumer's acceptability of value-added fishery product under modified atmosphere packaging was investigated in Dhaka and Rajshahi city by survey method utilizing structured questionnaire. Total 50 respondents, selected by voluntary response sampling method from each of the stores were surveyed (Table 68).

Table 68. Consumer's acceptability of value-added fishery products with MAP packaging (n=50)

Sl No.	Variables	Category	Frequency	Percent
1	Product name	Fish ball (Tilapia)	8	16.0
		Fish ball (Tuna)	4	9.0
		Fish Sausage (Thai Pangas)	2	4.0
		Fish Sausage (Mackerel)	1	2.0
		Battered & breaded fish (Tilapia)	5	10.0
		Battered & breaded fish (W. snapper)	1	2.0
		Fish marinades (Sardine)	1	2.0
		Fish crackers (Tilapia)	18	36.0
		Fish crackers (Tuna)	10	20.0
2	Purchase location	Dhaka	13	26.0
		Rajshahi	37	74.0
3	Preferred fish type	Iced	36	72.0
		Frozen	14	28.0
4	Buying of Value-added meat products	Yes	33	66.0
		No	17	34.0
5	If Yes, name of products	Ball	3	9.1
		Sausage	6	18.2
		Nuggets	6	18.2
		Frozen Chicken	8	24.2
		Sausage + Nuggets	3	9.1
		Ball + Nuggets + Sausage	7	21.2
6	Buying of value-added fish product	Yes	4	8.0
		No	46	92.0
7	If Yes, name of products	Fish Sausage	2	50.0
		Fish finger	1	25.0
		Fish ball + fish finger	1	25.0
8	Preference on buying value-added products	Chilled/Refrigerated	13	26.0
		Frozen	36	72.0
		Both	1	2.0
9	Find chilled value-added products in market	Yes	17	34.0
		No	33	66.0
10	Preference on buying fish products	Fresh water fish	33	66.0
		Marine fish	15	30.0
		Freshwater & Marine	2	4.0
11	Knowing about MAP packaging	Yes	39	78.0

Sl No.	Variables	Category	Frequency	Percent
		No	11	22.0
12	Liking of products packaging	Yes	48	96.0
		No	2	4.0
13	Preferred pack size	100	16	32.0
		150	6	12.0
		200	10	20.0
		250	14	28.0
		Others	4	8.0
14	Possibility of marketing of these value-added fish products	Yes	50	100.0
		No	0	0.0

Out of nine products, highest 36% responses came from Tilapia Fish crackers and 20% each from Tuna Fish crackers, 16% from Tilapia Fish ball; 10% from Battered & breaded Tilapia, 9% from Tuna Fish ball, 4% from Thai Pangas sausage and 2% each from other 3 products. The highest responses (74%) from Rajshahi city and rest from Dhaka city. In those outlets, 72% consumers purchase iced fishes, and rest 26% purchase frozen fishes. Around 72% respondents value-added meat products from the market and mainly sausage, ball and nuggets. Around 92% respondents don't buy value-added fishery products from the market and rest buy it which are mainly Fish sausage and fingers. The preferable type of value-added products was frozen by 72% respondents and rest were chilled or refrigerated. Most of the respondents (66%) didn't find any value-added products under chilled or refrigerated condition. The value-added fishery products from fresh water fishers were preferred by 66% respondents and marine fishes by 30% respondents. Around 78% respondents knew about MAP packaging which is very interesting., that is due the supplied leaflet along with products. The packaging of the products was preferred by 96 % respondents. Around 32% respondents preferred the 100g pack size and 28% preferred 250g pack size. The 100g pack size might be for crackers and 250g pack might be for other RTC products. The 100% respondents opined on the possibility of introducing this kind of MAP packaged value-added fishery products in the market. Therefore, there is a scope to produce MAP packed value-added fishery products which can be easily sell for a longer period either at refrigerated condition or room temperature.

11.2.5 Cost-benefit analysis of MAP packaged value-added fish products

A total of 9 value-added fishery products were prepared and packed under MAP packaging with suitable gas mixture having highest shelf life. Then all RTC and RTE products were sold out to the consumers in three outlets for studying consumer's acceptability of those value-added products. In this study, cost-benefit analysis was done to know the pricing of the products. The cost-benefit analysis of MAP packaged RTC and RTE products are shown in Table 69. By knowing this analysis, stakeholders (small or large scale) can take initiative to start their business based on this kind of fishery products which is much needed in our society now. In this study, pricing was set based on average of 26% profit margin for all products.

Table 69. The cost-benefit analysis of RTC and RTE value-added fish products under MAP packaging conditions

All values are in Tk

SL No	Inputs/ingredients	Fish ball		Fish sausage		B & B fish		Marinades	Fish crackers	
		Tilapia	Tuna	Pangas	Mackerel	Tilapia	W snapper	Sardine	Tilapia	Tuna
1	Fish flesh of 1 kg	525	375	416	360	600	920	169	525	375
2	Corn flours	14.4	14.4	57.6	57.6					
3	Spices	22	22	9	9	21	21	9		
4	Breadcrumbs	18	18	0	0	18	18			
5	Eggs	16	16	16	16	16	16			
6	Tapioca flour								121	121
7	Wheat flours			11.2	11.2	10.5	10.5			
8	Oils								300	300
9	Vinegar							3.2		
10	Sugar									
11	Salt	0.3		0.88	0.88	0.6	0.6	0.0015	2.4	2.4
12	Lemon	3	3	3	3	6	6			
13	Sodium bicarbonate								8.8	8.8
14	Labor cost	125	125	95.625	95.625	160	160	55	150	150
15	Packaging cost	50	50	50	50	55	55	35	120	120
16	Gas cost	50	50	50	50	55	55	35	300	300
17	Transport & Storage cost	20	20	20	20	22	22	14	180	180
	Total cost in TK	**843.7**	**693.4**	**729.3**	**673.3**	**964.1**	**1284.1**	**320.2**	**1707.2**	**1557.2**
	Yield in g	**1250**		**1530**		**1600**		**1100**	**1500**	
	Weight per pack (g)	**120**		**150**		**150**		**150**	**25**	
	Cost per pack in Tk	84.37	69.34	72.9	67.3	87.6	116.7	29.1	28.5	26.0
	Market Price per pack in Tk	100	100	90	90	110	150	60	35	35
	Total income in Tk	**1000**	**1000**	**900**	**900**	**1210**	**1650**	**420**	**2100**	**2100**
	Total profit in Tk	156.3	306.6	170.7	226.7	245.9	365.9	99.8	392.8	542.8
	Profit in percentage in Tk	**15.6**	**30.7**	**19.0**	**25.2**	**20.3**	**22.2**	**23.8**	**18.7**	**25.8**
	Average profit (%) in Tk	**25.8**								

12. Research highlight

12.1 . Component-1 (NSTU)

Title of the sub project: *Development of fish-based food products and extension of shelf life to enhance nutritional security*

Research highlights 01
Nutrient content of Fish powder of five fish species

Background
Fish is a unique animal-derived food in terms of supplying protein in the diets of millions of people in Bangladesh. Many factors, for example, globalisation, urbanisation, the increasing numbers of working women, high disposable incomes, and media penetration, are causing a change in lifestyles, including the increasing use of processed foods. Additionally, many children are used to eating snack food items, including biscuits, soup, and burgers, and avoid raw fish in their regular diet due to the excess number of Y-bones and hard spiny bones. In this regard, Ready-to-use fish products (RUFPs) are gaining popularity among consumers as supplementary novel food products and in the private sector for their diverse range of applications in the food industry. Therefore, the present study aimed to develop Fish powder from three freshwater species (Punti, Chapila, Kachki) and two marine species (Anchovy and Sardine) and analyse their nutritional quality: the proximate composition and minerals and the fatty acid and amino acid profile to provide data for researchers, policymakers, consumers, and the food industry. In addition, the contribution of the RUFPs to recommended nutrient intake (RNI) was assessed to evaluate their contribution to food and nutrition security.

Objective
To know the intra-household food consumption, food allocation and nutritional outcomes of adolescent girls through baseline survey.

Methodology
Three low-cost, nutrient-dense fish were selected and collected for the preparation of RUFPs. Indian river shad (Chapila, *Gudusiachapra*) and Ganges river sprat (Kachki, *Coricasoborna*) were collected from the Kaptai Lake, Rangamati, whereas Ticto barb (Tit punti, *Puntius ticto*) was collected from the haor areas of Kishoreganj. Pointed tail Anchovy (*Coiliadussumieri*) and fringe scale Sardine (*Sardinella fimbriata*) deceased fish samples were purchased from the fish landing centres of Cox's Bazar, Bangladesh.

Fish samples were divided into three groups for the development of the whole, gutted, and muscle RUFPs. For the development of powder from whole fish, samples were completely washed with water to eliminate dirt, dust, and other foreign matter. Then, whole fish, gutted fish,

and muscle was subjected to sun and oven drying. The ground powder was packaged with high-density polyethylene and stored at 4ºC for nutritional analysis. The proximate composition (moisture, protein, lipid, ash, and carbohydrate), mineral (sodium, magnesium, phosphorus, potassium, calcium, chromium, manganese, iron, cobalt, copper, zinc, selenium, arsenic, cadmium, lead, vanadium, and nickel), amino acid profiles and fatty acid composition of the developed powders were evaluated.

Key findings

The quantitative yield among the five species, anchovy produced the most powder (19.05%) followed by sardine (15.25%), Chapila (13.65%), Kachki (12.81%), and Punti (11.25%). The moisture content was highest in the RUFPs developed from whole fish, followed by gutted fish and the fish muscle.The protein content was found to be significantly higher in the powder developed from muscle than that of gutted and whole fish. The highest lipid content was in the whole Punti powder (17.58 ± 0.10%) and the lowest lipid content was in the anchovy muscle powder (6.42±0.06%). The highest amount of carbohydrate was determined in the whole sardine powder (9.19±0.07%) and the lowest was in the whole Chapila powder (2.54 ± 0.04%). The sodium content was highest in the gutted anchovy powder (4998.73±18.82 mg/kg) and lowest in the Chapila muscle powder (1859 ± 27.81 mg/kg). The highest amount of chromium was found in the whole Sardine powder (10.06±0.20 mg/kg) and the lowest was found in whole Kachki powder (0.30 ± 0.03 mg/kg). The highest level of zinc was found in the whole Kachki fish powder (128.25 ± 0.69 mg/kg) and the lowest in the Anchovy muscle powder (66.01±1.04 mg/kg). The amounts of threonine, valine, methionine, isoleucine, leucine, phenylalanine, tryptophan, and lysine were highest in the RUFPs developed from the whole Punti. Among all the fish powders, the highest amount of PUFA was found in the whole Sardine powder (2619.79±0.86 mg/100 g) and the lowest was found in the Anchovy muscle powder (841.73±4.19 mg/100 g). The EPA + DHA value was the highest in the whole Sardine powder (1303.69 mg/100 g) and lowest in the Punti muscle powder (237.14 mg/100 g).

The present study indicates sun drying is a potential method for developing whole fish, gutted fish, and muscle powder of Punti, Chapila, Kachki, Anchovies, and Sardines. Based on proximate composition, developed Fish powders are rich protein sources; therefore, Fish powders would be useful for developing a Protein-rich diet for vulnerable populations. Additionally, fish powders are novel sources of minerals, especially K, Ca, Fe, Zn, and Se. Furthermore, the developed RUFPs are also high in EAA, NEAA, EPA, DHA, and n-3 and n-6 fatty acids. They can also be used as food aids in conflict areas such as the Rohingya refugee camp and the vulnerable group feeding (VGF) program during fish banning periods and also play a key role to diminish hidden hunger.

Keywords: Fish powder, Proximate composition, Mineral compositions, EPA+DHA composition, Amino acid composition

Research highlights 02

Randomized control trial (RCT) of Fish powder among adolescent girls

Background

Fish and fishery products are the major sources of animal origin diets of the people. Ready-to-eat fish (prepared status of fish and fish products for consumption) based products like Fish powdercould be a very good option to mitigate many challenges of food and nutritional security of the vulnerable population. These products could be an alternative choice to utilize during the lean period, as food aid for the crisis population (for instance *Rohingya* immigrants from Myanmar, *Monga;* seasonal food crisis-affected people in northern Bangladesh). Moreover, incorporating this powder into local cuisine will make it popular in all segments of society. Food preference has been changing with the social and economic development of the country. Now, city-dwellers especially busy mothers and housewives seek Ready-to-cook (RTC) or prepared foods instead of raw ingredients in their busy life. Presently, there is no such value-added fishery product in the market. However, recently several companies in the country introduced some value-added meat products under the frozen condition in the market. The current study evaluated the efficacy of Fish powder among adolescent girls.

Methodology

Households from each social well-being group were selected purposely, and the criteria were selected households having at least one unmarried adolescent girl (10-19 years old). On this ground, at least 40 households from each community (40 HH×2 arms [intervention, control] ×3 [areas; Noakhali, Lakshmipur, and Khagrachari=240 HH) were selected. Intra-household food allocations and nutritional outcomes of the adolescent girls are derived from the in-depth survey. A 24-hour food recall method and food frequency questionnaire were developed and validated accordingly. Anthropometric data and blood samples were collected in a health center, developed as temporary sample collection centers on the same day. The end-line survey was conducted at the same sites and a similar level of data has been collected. In this phase, about 10% of girls were missing as they got married in the meantime and/or were absent in their household during the survey time. All girls were given a token gift for their participation same as the baseline survey. Households at the intervention site got token money each week for their participation during the RCT.

Objective

Development of ready to use fish-based nutrition-dense products using most suited fish species on the basis of nutritional quality, affordability and availability and assesses their quality, appearance and microbial load.

Key findings

Vitamin B12 was found higher in the 2nd phase than in the 1st phase. However, average Calcium was higher in 1st Phase followed by CRP and others. Vitamin B12 was found significantly high

in intervention areas which may help to focus the project on an important thing Fish powdercan increase vitamin B12 Among adolescent girls. Except for hemoglobin, overall anthropometric parameters were higher in the second phase than in the 1st phase. BMI of sampled individuals of Noakhali (intervention and control) was higher than in the rest of the regions. On average, glucose, hemoglobin, BMI, and MUAC increment were found in the intervention than in control of the Noakhali region. However, average weight and height became high in control than in intervention. A minimal level of increment was found in average BMI, MUAC, glucose, and hemoglobin of intervention and control of the Lakshmipur region. However, blood pressure was found higher along the control areas for this reason. However, BMI, MUAC, and Glucose showed an increment in control rather than intervention samples. It was found that except Ki, vitamin B12 in all interventions increased in the second phase rather than in the first phase. All CRP values are in the normal range. It shows that except for Li, calcium increased in all sampling areas. This is indicating the iron deficiency of people in the collected samples. High glucose, ranges were found in sampling areas. It shows the normal ranges of hemoglobin from the sampling area. With several outliers, it was found that vitamin B12 increased from fast fare to the second phase in each study area. The range of a normal vitamin B12 in the human body. It seems that most adolescent girls have a normal range of vitamin B12 accordingly and it increases after a diet fortifying with Fish powder.Except for a few cases, all ferritin levels of adolescent girls are in normal ranges. However, it decreased in the 2nd phase compared to the 1st Phase. Notably, it decreased sharply in control areas from study regions.Iron deficiency was found in most of the adolescent girls which were indicated by the boxplot crossed through the low ranges of normal iron amount, especially in Lakshmipur (control). Average increments of iron are found in the second phase of intervention areas. Fish powder consumption tendency was higher in Lakshmipur and lower in Khagrachari. On the other hand, moderate FP consumer was higher in Noakhali. After 16 weeks of intervention, it was found that high consumption frequency increased vitamin B12 levels in the serum of the blood of adolescent girls in those areas, especially in Noakhali. Some outliers indicate the fascination of respondents with eating dry fish. Cereals and fish consumption were higher in all areas, Leafy vegetable consumption was found low than other vegetables. Among all the biomarkers, it indicated that vitamin B12 has shown a clear increment after the intervention period of time. Fish powder consumption among adolescent females in intervention households was found satisfactory level.

Keywords: Adolescent female, Ready-to-use fish products, Fish powder, Randomized control trial

Research highlights 03

Biological evaluation of the alterations in serum level of Vit-B12, Ferritin, CRP, Calcium, and Iron level after providing Fish powder supplement in anemic mice- a case-control study

Background

The deficiency of trace elements and vitamins may responsible for the major nutritional disorder in low and middle-income countries including Bangladesh. Children are a particularly sensitive

population to diseases like anemia, starvation, and other fatal ones. As dry Fish powdersupplements possess high protein and nutrients, they can be a good source for combating the above disease. The objectives of this study are to investigate the effect of Fish powdersupplements on anemic mice to treat the anemic condition, and also to investigate the effect of the fish supplement on biomarkers such as vitamin B12, ferritin, C-reactive protein (CRP), calcium, and iron level using Swiss albino mice.

Objective

Screening the best suited fish-based products on the basis of different nutritional, microbial, toxicology and sensory evaluation and fit in the best products into the local cuisine with an appropriate ratio of fish and vegetable/legumes to minimize the nutritional gaps obtained from the baseline survey.

Methodology

At first, we purchased the female Swiss albino mice aged 35-40 days from icddr'b. These mice were kept in the laboratory condition for 7 days at *ad-libitum*. After adaptation, the body weight, and hemoglobin levels of the mice serum were recorded. Later these mice developed iron deficiency anemia by losing blood from the tail using the blood loss method. When the anemic condition of mice was confirmed, we collected blood samples from mice, centrifuge those, and later stored the serum for further analysis. Approximately 100 mL serum per mouse were collected for the analysis (baseline data). After collecting the serum, 28 days intervention period started; during this time Fish powder supplements were given to the mice. After completing the intervention period, blood samples were collected once again which was followed by centrifugation and collection of the serum. Initial and final serum samples were sent to icddr'b for further analysis.

Key findings

It was found that serum Vit B12 and calcium conc. level increases approximately 10-20% due to the consumption of Fish powder supplements by the mice. Moreover, Ferritin levels increase significantly (approximately 40-50%, $p<0.05$) in the mice serum after providing Fish powder supplements.

We did not find any changes in CRP and Iron levels due to the introduction of Fish powderto the feed plan of anemic mice. Providing dry Fish powderin the meal chart of the mice may significantly increase the serum trace elements and vitamin level, which indicates that Fish powder may have high potency to combat malnutrition and vitamin deficiency in adolescent children.

Keywords: In-vivo, Mice, Vitamin B12, Fish powder, Malnutrition

Research highlights 04

Consumer acceptability test of Fish powder

Background

Value-added products either Ready-to-cook (RTC) or Ready-to-eat (RTE) fishery products with key nutrients could be introduced into the market. Fish is usually sold as a whole or cut form without proper storage and display facilities. As a result, a considerable amount of raw fish undergoes quality deterioration resulting in quantitative loss during retailing. Both RTC and RTE fish-based products could be an alternative option for a sustainable food management.The main objective of the project is to know the consumer preference to use and buy Fish powder. The work is designed to know the household preference level of Fish powderconsumption, and the pattern of using Fish powder.

Objective

To evaluate the effect of nutrient-rich Chapila fish supplement to mitigate anaemia in mice model.

Methodology

The project used a survey-based methodology to collect data. Noakhali municipality households were selected for collecting data. A semi-structured questionnaire was developed and used to collect data. It was directed to the surveyor to provide Fish powderto their familiar family because of the Covid-19 lockdown restriction. To collect data 100 sample products (each sample product containing 250 grams of Fish powder) were distributed to 100 households (minimum of 4 members). We fixed 10 days for each household to eat this Fish power. A total of 401 respondents were found, out of which, 249 responses were completed and valid for the analysis after removing incomplete and unusable responses. The respondents were asked to share their views on the taste, effectiveness, usability, benefits, and disadvantage of the Fish powder. Data were analyzed through Microsoft excel by using graphs, pie charts, column charts, tables, and word clouds to get the findings of the study.

Key findings

Findings revealed that 50% of people were fully satisfied with the product's color, smell, and taste, whereas 40% of people were neutral. 55% found Fish powdersafer than dry fish and 47% of the respondents feel Fish powderis safe for children. Now, most importantly 56% of the respondents want this product and 37% are neutral, which is good. Most of the respondents close to 50% want the product in the price range of 100 Tk to 200 Tk. They want to buy the product in 250-gram packets in quantity. Through the SWOT, the respondents feel that the strength of the product is "less time", "nutritious "," thorn less", " easy cooking" and the weakness of the product can be its smell and price a very good opportunity in urban areas and it can have the threat of competition and formalin. Prepared Ready-to-use Fish powderwas well accepted by the studied household. The overall score of the Fish powderwas at a satisfactory level and indicated a prospective of this product.

Keywords: Ready-to-use Fish powder, Household, Consumption, Preference

Research highlights 05

Shelf-life prediction of Ready-to-cook Chapila (Gudusiachapra) Fish powder storage at 45°C and 55°C

Background

Shelf-life is an important feature of food, and can be defined as the length of time that the food products could be stored without their quality becoming unacceptable to consumers. In general, real-time storage will take a long time to determine the shelf life of food, but accelerated storage can easily shorten the experimental period (Mizrahi, 2000). According to the State Food and Drug Administration of China, peroxide value (POV) is selected as the indicator to evaluate the shelf life of dried aquatic products, and the acceptability limit was 47.28 meq/kg fat. However, to the best of our knowledge, no study regarding the shelf-life prediction of Fish powderthrough accelerated storage has been reported. Thus, the objective of this study is to investigate the changes in fatty acid profiles and shelf-life estimation of Chapila Fish powderduring accelerated storage. To fulfill this objective, proximate composition, minerals content, fatty acid profiles, pH, TVBN, and peroxide value in the Chapila Fish powderduring accelerated storage were determined.

Objective

To know consumer satisfaction and acceptability of using Fish powder for cooking.

Methodology

Chapila (*Gudusiachapra*) Fish powder samples packed with zipper and vacuum pack were collected from the Department of Fisheries and Marine Science, Noakhali Science and Technology University, Noakhali-3814. Then the samples were kept at 45°C and 55°C for up to 45 days for analysis to predict the shelf-life of Ready-to-Cook Chapila Fish powder. The moisture content of Chapila Fish powderwas determined by evaluating the loss of weight after drying the sample in a hot air oven at 105 °C overnight until the weight became stable (AOAC, 1990).Ash content was determined in moisture-free dry samples in a muffle furnace at 550 °C for 20 h until all organic components of the sample were incinerated completely (AOAC, 1990). The crude protein content of Tilapiasamples was determined by multiplying the nitrogen content obtained by the Kjeldahl method by the conversion factor of 6.25 (AOAC, 1990). The crude lipid content of the Tilapia sample wasdetermined by extracting fat using the Soxhlet method (AOAC, 1990).To determine the mineral content, Chapila Fish powdersamples were dissolved and digested with HNO_3 at 200 °C for 2 h (AOAC, 1995). Phosphorus (P) and iron (Fe) contents in Fish owdersamples were determined by the atomic absorption spectroscopy method using the atomic absorption spectrophotometer model AA-7000 (Gokoglu et al. 2004). The pH value of the Chapila Fish powderwas determined by using a pH meter (Mettler Toledo 320-s, Shanghi, China) followed the standard method (Vyncke, 1981). The TVBN value was measured by using

Conway's modified micro-diffusion technique (Conway and Byrne, 1933). The peroxide value was calculated according to the American Oils Chemists' Society official method AOCS Cd 8-53 (AOCS, 1998). Total lipids and fatty acids are extracted from Fish powderby the hydrolytic method. The fatty acids were obtained from saponification of total lipids of Fish powdersample with NaOH and were converted into fatty acid methyl esters (FAMEs) by methylation using HCl and methanol mixture. The composition of fatty acids in Chapila Fish powderwas determined by the gas chromatographic (GC) method (Kocatepe and Turan, 2012). The comparison of the retention times and peak areas with the respective peak areas of the relevant fatty acids' standards was used to identify and quantify the individual fattyacids (Mohanty et al. 2019).

In the present study, the desired expiration date of the developed Fish powder is one year, and the test temperature was selected at 45°C and 55°C, Q10 is 2; the accelerated aging time duration is determined as follows:

A1 = 2((45-25)/10) = 4

A2 = 2((55-25)/10) = 8

B1 = 365 days/4 = 91 days

B2 = 365 days/8 = 45 days

Key findings

The proximate composition, mineral content, pH, TVBN, and peroxide value. The TVBN and peroxide values are increased gradually during the accelerated storage period. The lipid oxidation and degradation occurred in Chapila Fish powderduring accelerated storage, which was reflected by the increase in all oxidation indices including peroxide value, TVBN, and PUFA percentage. By calculation, temperature co-efficient (Q_{10}) of shelf-life at 45°C and 55°C the actual accelerated aging time duration values were 91 days and 45 days, respectively which demonstrated developed Chapila Fish Powdershelf-life to be one year due to the peroxide value and TVBN value are expectable range during the accelerated storage periods. Furthermore, the study also reported that hapilaFish powderin zipper bag packaging deteriorates faster than in vacuum packaging as well as the deterioration rate is closer at 55°C than 45°C.

Keywords: Shelf life, Ready-to-use Fish powder, Fish powder, Bangladesh

12.2 Component-2 (RU)

Research highlights 01

Development of RTC and RTE products from freshwater and marine fish.

Background

Food preference has been changing with social and economic development of the country. Now, city-dwellers especially busy mothers and housewives seek Ready-to-cook (RTC) or prepared

foods instead of raw ingredients in their busy life. Presently, there is no such value-added fishery products in the market. However, recently several companies in the country introduced some value-added meat products under frozen condition in the market. On the other hand, with increasing consumer demands for fresh products with extended shelf life and increasing energy costs associated with freezing and frozen storage, the fish-processing industry is actively seeking alternative methods of shelf life preservation and marketability of fresh, refrigerated fish and at the same time economizing on energy costs (Ashie*et al*. 1996).Therefore, value added products either Ready-to-cook (RTC) or Ready-to-eat (RTE) fishery products with sufficient shelf-life under refrigerated condition could be introduced in the market.

Objectives

Development of Ready-to-cook (RTC e.g. Fish ball, Battered &breaded fish, Fish marinades, Fish sausage) and Ready-to-eat (RTE e.g. Fish crackers) from freshwater and marine fish.

Methodology

Both marine and freshwater fishes were used for preparation of Ready-to-cook and Ready-to-eat products. Freshwater fishes were bought from the farm/market and brought to the Quality Control Laboratory of the Department of Fisheries in the University of Rajshahi under live condition. On the other hand, marine fishes were collected from marine fish landing center in Chattogram or from a commercial fishing vessel as frozen and brought to the Department of Fisheries in Rajshahi University under icing condition. After arriving, freshwater fishes were used directly for product development and marine fishes was kept in frozen storage at -18°C for further processing. Tilapia (*Oreochromis niloticus*) was used for the preparation of Fish ball, Battered &breaded fish, and Fish crackers and Thai Pangas (*Pangasianodonhypophthalmus*) was used for the preparation of Fish sausage. On the other hand, Skipjack (*Katsuwonus pelamis*) was used for preparation of Fish ball &Fish crackers, Mackerel (*Rastrelligerkanagurta*) was used for preparation of Fish sausage and white Snapper (*Lutjanus johnii*) was used for Battered &breaded fish. In addition, Sardine (*Sardinella fimbriata)* fish was used for the preparation of Fish marinades. By optimizing the amounts of ingredients and different processing parameters, all of those nine value-added products under five categories were developed and make a standard recipe of each product(Categorywise preparation method of each product are presented as follows):

i. Preparation of Fish ball

Ingredients required*

- Corn flour- 40 g; Ginger paste-10 g; Garlic paste- 8 g; Corriander-2.5g; Cumin-2.5g; Red chili powder- 3 g; Black pepper powder-1.5 g; Soy sauce- ½ tsp; Lemon juice- 2 tsp; Salt- 7.5 g; Onion-40g; Green chili-4 pcs and Bread crumbs- ½ cup;

Breaded ingredients**

- Egg-2 and Bread crumbs- ½ cup

Preparation process

The fishes were washed, beheaded and eviscerated. The skinned fishes were filleted and deboned manually under chilled condition. The minces were prepared by a mechanical meat mincer (Panasonic Corporation, Japan) through a 1mm orifice diameter so that all bones and connective tissues was removed from the muscles.

First 500 g of fish mince was taken in a bowl and then added different ingredients* and mixed well. The mixture was then kept at refrigerator for 30 min. Then made round shaped ball manually. The prepared ball was then soaked in beaten eggs and rolled on bread crumbs**. Fish ball was then ready for MAP packaging and preservation at 4°C.

ii. Preparation of *KeropokLekor* type Fish sausage

Ingredients required

- Corn flour-160 g; Wheat flour-80g; Sugar-14g; Salt- 11g; Ice-60-100g; Pepper- 2g; Chilli powder- 1.5g and Soya sauce-1/2 tsp

Preparation process

Fish (Pangas/Mackerel) mince was prepared similar to that of Fish ball preparation.First 500g of fish mince was taken in a bowl and then added different ingredients (as stated)* and mixed well. The mixture was entered into the sausage stuffer to prepare fish sausage. Then the sausage was boiled at hot water until floated. Fish sausage was then ready for MAP packaging and preservation at 4°C.

iii. Preparation of Battered & breaded fish

Ingredients required*

- Ginger paste-10 g; Garlic paste- 10 g; Cumin powder- 2 g; Coriander powder-2 g; Red chili powder- 4 g; Turmeric powder-2 g; Soy sauce- ½ tbs; Lemon juice- 2 tsp and Salt- 7 g.

Breaded ingredients**

- Wheat flour- 1 cup with cumin 2 g, Red chili- 2 g, Salt; Egg-2 and Bread crumbs-1 cup

Preparation process

Fishes (Tilapia/white Snapper) were filleted first and kept aside. Different ingredients were mixed well in a bowl. The mixture of ingredients was rubbed well on the fillets and kept at the refrigerator for 1h. After that, the fillets were rolled on flour with other ingredients* *, soaked in beaten eggs and finally rolled on bread crumbs. Then the products were kept at refrigerator for 30 min with plastic wrap. Then the Battered and breaded Fish fillets were ready for MAP packaging and preservation at 4°C.

iv. Preparation of Fish marinades

Soaking Solution*

- Acetic acid-2% and Salt-10%

Others Ingredients**

- Garlic-7.5g; Ginger-5 g; Red chili-2.5 g; Coriander-1.5 g; Turmeric- 1.5g and cumin-1 g

Preparation process

Sardine fishes were cut into slices and washed with water. First 500 g of fish slices was taken in a bowl and added 10% salt and 2% acetic acid solutions. Then stored at refrigerator (4°C) for 48 h. The fish slices were taken out and drained off the solution. Then different ingredients* were added and mixed well. Then Fish marinades were ready for MAP packaging and preservation at 4°C.

v. Preparation of Fish crackers

Ingredients*

- Tapioca flour-100 g; Sugar-6 g; Salt-5.5 g; Ice-15 g, and Sodium bicarbonate-1 g

Preparation process

Fish (Tilapia/Tuna) mince was prepared similar to that of Fish ball preparation. First 90g of fish mince was taken in a bowl and added ingredients* and mixed well. The dough was shaped into cylindrical and steamed for 30 m followed by chilling in refrigerator overnight. On the next day, cylinder shaped product was cut into 2 mm thickness and put into oven of 50°C for 10 to 12 hours until the moisture content reading was 10% ± 2%. Dried crackers were then deep fried in vegetable oil at 170-180°C. Then the Fish crackers were prepared asReady-to-eat (RTE) products for MAP packaging and preservation at room temperature.

Key findings

Standard Recipe of 9 value-added fishery products has been developed. The RTC products were Tilapia Fish ball, Tuna Fish ball, PangasFish sausage, Mackerel Fish sausage, Battered &breaded Tilapia fillet, Battered &breaded white Snapper fillet, Sardine Fish marinades and RTE products were Tilapia Fish crackers and Tuna Fish crackers. The products contained protein in the range of 11-19% where highest 19.32% protein found in Sardine Fish marinades and lowest in 10.93% in tuna Fish crackers.

Key words: Recipe, Fish ball, Fish sausage, Battered &breaded fish, Fish marinades, Fish crackers.

Research highlights 02

Shelf-life of RTC and RTE fish products

Background

Value added products either Ready-to-cook (RTC) or Ready-to-eat (RTE) fishery products with sufficient shelf-life under refrigerated condition could be introduced in the market. Modified atmosphere packaging (MAP) combined with low storage temperatures is an effective technique to achieve shelf-life extension of fishery products (Sivertsvik*et al*. 2002). MAP is a widely used packaging technique for displaying chilled fish, meat and their products in developed countries. It extends the shelf-life (25-400%) of the raw fish fillets at refrigeration temperature (Reddy *et al*. 1991; Pantaza*et al*. 2008). It allows retailers to sell raw fishes and their products at refrigeration condition for an extended period. In our previous study, the highest shelf-life of

sliced tilapia was 15 days for MAP 1 (50%CO_2 & 50%N_2) followed by 12 days for MAP 2 (50% CO_2 & 50% O_2), 9-12 days for vacuum pack and 6-9 days for no pack (control) sample (Karim et al. 2020). In case Rohu fish, the highest shelf-life was 16 days for MAP 1 (50%CO_2 & 50%N_2) followed by 13 days for MAP 2 (50% CO_2 & 50% O_2), 11 days for vacuum pack and 8 days for no pack (control) sample (Das et al. 2021). On the other hand, the highest shelf-life of sliced Goonch fish (Baghair) was 12 days for MAP 1 (50%CO_2 & 50%N_2) followed by 10 days for vacuum pack, 9 days for MAP 2 (50% CO_2 & 50% O_2), and 6 days for no pack (control) sample (Alice et al. 2020). However, vacuum packaging significantly increased the shelf-life, but it is not suitable for all types of fishery products particularly for soft and breakable products, as the pressure created during vacuum packaging can break down the products structure inside the pack. Therefore, MAP packaging with thermoformed tray (using Tray Sealer) instead of pouch is more convenient for displaying the products for the consumer. Previously, several scientists attempted to develop different types of value-added fishery products in the country including Fish ball, Fish finger, Fish mince, Fish burger, Fish cutlet etc. which can be stored at freezing and refrigeration temperature (Hoque et al. 2007,Nowsad et al. 2007, Flowra, 2013, Zzaman*et al*. 2017). However, no study was found to increase the shelf-life of those value-added products by combination of MAP packaging and refrigerated storage condition. Therefore, there is a scope to produce different value-added products from low value fish and preserve those fishery products under MAP packaging condition in different storage condition to get the sufficient shelf-life for marketing in the superstores.

Objectives

To determine the overall shelf-life of RTC and RTE products stored under modifiedatmosphere packaging at different storage temperature.

Methodology

The RTC fish products (Fish ball, Battered & breaded fish, Fish marinades and Fish sausage) were packed in tray (PP/EVOH/PP; 760 µm) with plastic top (PE/PA/PE; 100 µm). Four types of packaging were applied under modified atmosphere packaging with different gas ratio using the method described by Noseda et al. (2012) with some modifications using a tray sealer (Oceania mini, Italian Pack, Italy) attached with Gas Mixer KM100-3 MEM (WITT, Germany). Analysis of the O_2, N_2 and CO_2 levels in the headspace of the packaged samples were performed with Oxybaby M+ gas analyzer (WITT, Germany). Those four types of packaging were used as treatments namely, (a) aerobic, not sealed as control; (b) MAP 1 (50%CO_2 & 50%N_2) as treatment-1; (c) MAP 2 (40% CO_2, 30% N_2 & 30% O_2) at treatment-2 and (d) MAP 3 (50% CO_2 & 50% O_2) as treatment-3. On the other hand, RTE fish products were packed in plastic film (PE/PA/PE; 100 µm) performed in MAP packaging machine (C100, Multivac, Germany). Three types of packaging were used as treatments namely, (a) air pack as control; (b) MAP 1 (100% N_2) as treatment-1; and (c) MAP 2 (50% CO_2 & 50%N_2) as treatment-2. The RTC and RTE products were stored at 4˚C and room temperature, respectively. The packaged products in triplicate were subjected to biochemical and microbiological analysis (pH, Free Fatty Acids (FFA), Total volatile base nitrogen (TVBN), Thiobarbituric acid reactive substances (TBARS),

Aerobic plate count (APC)) in 4-10 days interval for RTC and a month interval for RTE products in the laboratory. One-way ANOVA with the application of Tukey test using SPSS Version 20 at $P < 0.05$ level of significance.

Key findings

In this study, the highest shelf life of Tilapia and tuna Fish ball were 20 and 16 days, respectively under MAP-3 (50% CO_2 & 50% O_2). MAP-1 (50% CO_2 & 50% N_2) demonstrated the highest shelf life of 26 days for both Pangas and mackerel Fish sausage. MAP-2 (40%CO_2, 30% N_2 & 30% O_2) gave the highest shelf life of 16 days for both Battered &breaded Tilapia and white Snapper. Fish marinades showed the 34 days shelf life under MAP-2 (40%CO_2, 30% N_2 & 30% O_2). Tilapia and Tuna Fish crackers demonstrated the highest shelf life of 5 and 6 months, respectively under both MAP-1 (100% N_2) and MAP-2 (50% CO_2 & 50% N_2). Therefore, these MAP packaging can be used to increase shelf life of RTC and RTE fish products.

Key words: Shelf-life, MAP, Tray sealer, TC, RTE, Temperature, Packaging

Research highlights 03

Consumer's acceptability of MAP packaged fishery products

Background

Food preference has been changing with social and economic development of the country. Now, city-dwellers especially busy mothers and housewives seek Ready-to-cook (RTC) or prepared foods instead of raw ingredients in their busy life. Presently, there is no such value-added fishery products in the market. Therefore, value added products either Ready-to-cook (RTC) or Ready-to-eat (RTE) fishery products with sufficient shelf-life under refrigerated condition could be introduced in the market. Modified atmosphere packaging (MAP) combined with low storage temperatures is an effective technique to achieve shelf-life extension of fishery products (Sivertsvik*et al*. 2002). It allows retailers to sell raw fishes and their products at refrigeration condition for an extended period. Therefore, development of different value-added products either RTC or RTE and preservation through MAP packaging and refrigerated storage is required to ensure the supply of quality fishery products with the extended shelf life in the market. However, consumer's acceptability and willingness are essential to study before introducing any new type of products in the market. Therefore, consumer's acceptability of the prepared MAP packaged value-added fishery products needs to be assessed in field situation particularly in retail superstores.

Objectives

To know the consumer's acceptability of MAP packaged fishery products in the superstores of the Dhaka city.

Methodology

Consumer's acceptability and willingness to buy the packaged RTC and RTE fish products was investigated by survey method utilizing a structured questionnaire. Purposive sampling technique was used for selecting three locations of stores in Amana big Bazar, Rajshahi; RU souvenir shop, RU campus; BARC canteen, Farmgate, Dhaka for data collection. Then, prepared and packaged RTC and RTE fish products were displayed in three retail stores. Consumer's acceptability of the supplied modified atmosphere packaged RTC and RTE fishery products was investigated by survey method utilizing structured questionnaire. The number of respondents selected by voluntary response sampling method from each of the stores were surveyed. The fill-up questionnaires were collected by post, courier, email, WhatsApp and directly etc. after eating the purchased products at home. The questionnaire was organized to investigate the consumer's socio-economic condition and preferences and acceptability of MAP packaged RTC and RTE fishery products on different aspects including organoleptic characteristics, price, pack size, quality and overall attraction of the products. Then, all the surveyed data was tabulated and subjected to descriptive analyses by using SPSS Version-20.

Key findings

A total of 43 shopper/consumers were responded until now to send back their opinion through supplied questionnaires. Out of nine products, highest 40% responses came from Tilapia fish crackers and 16% each from Tuna fish crackers and Tilapia fish ball; 9% each from Tuna fish ball and battered & breaded Tilapia and 2% each from other 4 products. The highest responses (86%) from Rajshahi city and rest from Dhaka city. In those outlets, 67% consumers purchase iced fishes, and rest 33% purchase frozen fishes. Around 75% respondents purchase value-added meat products from the market and mainly sausage, ball and nuggets. Around 93% respondents don't buy value-added fishery products from the market and rest buy mainly fish sausage and fingers. The preferable type of value-added products was frozen by 72% respondents and rest were chilled or refrigerated. Most of the respondents (65%) didn't find any value-added products under chilled or refrigerated condition. The value-added fishery products from fresh water fishers were preferred by 63% respondents and marine fishes by 33% respondents. Around 79% respondents knew about MAP packaging which is very interesting., that is due the supplied leaflet along with products. The packaging of the products was preferred by 95 % respondents. Around 35% respondents preferred the 100g pack size and 32% preferred 250g pack size. The 100g pack size might be for crackers and 250g pack might be for other RTC products. The 100% respondents opined on the possibility of introducing this kind of MAP packaged value-added fishery products in the market. Therefore, there is a scope to produce MAP packed value-added fishery products which can be easily sell for a longer period either at refrigerated condition or room temperature.

Key words: Consumers, Respondents, Preference, Pack size

B. Implementation status

1. Procurement (component wise)

Coordination component (BARC)

Description of equipment and capital items	PP Target		Achievement		Remarks
	Physical (No.)	Financial (Tk.)	Physical (No.)	Financial (Tk.)	
(a) Office equipment • Procurement of Photocopier	01	398000.00	01	398000.00	Completed as per plan
(b) Lab & field equipment					
(c) Other capital items					

Component-1 (NSTU)

Description of equipment and capital items	PP Target		Achievement		Remarks
	Physical (No.)	Financial (TK.)	Physical (No.)	Financial (TK.)	
a. Office equipment		**450000**		**450000**	100% achievement
i. Laptop	2	120000	2	120000	
ii. Laser Printer	1	20000	1	20000	
iii. Table	6	120000	6	120000	
iv. DSLR Camera	1	55000	1	55000	
v. Video camera	1	80000	1	80000	
vi. Multimedia Projector	1	55000	1	55000	
b. Lab & field equipment		**796000**		796000	Achievement 100%
i. Freezer	1	80000	1	80000	
ii. Micro pipette	1	50000	1	50000	
iii. Ice boxes	1	20000	1	20000	
iv. Weighing and height measurement Machine	1	45000	1	45000	
v. Mini portable hammer mill	1	70000	1	70000	
vi. Tray oven dryer	1	200000	1	200000	
vii. Portable electric Vacuum sealer	1	12000	1	12000	
viii. Centrifuge Machine	1	55000	1	55000	
ix. Glucometer	1	24000	1	24000	
x. Digital Blood pressure machine	1	20000	1	20000	
xi. Blood withdrawing equipment	1	20000	1	20000	
xii. Food supplement weighing machine	1	30000	1	30000	
xiii. Chemicals	Lot	1,70,000	Lot	1,70,000	
c. Other Capital Items equipment	0	0	0	0	--

Component-2 (RU)

Description of equipment and capital items	PP Target		Achievement		Remarks
	Physical (No.)	Financial (TK.)	Physical (No.)	Financial (TK.)	
(a) Office equipment					
1. Desktop Computer	01	60000.00	01	46000.00	Completed as per target
2. Laptop	01	60000.00	01	80000.00	
3. Laser Printer	01	20000.00	01	19800.00	
4. Scanner	01	10000.00	01	7000.00	
5. UPS	01	10000.00	01	7000.00	
(b) Lab & field equipment					
1. Laboratory Refrigerator	01	200000.00	01	130000.00	Completed as per target
2. Tray Sealer	01	2200000.00	01	1748800.00	
3. Sausage Stuffer	01	35000.00	01	65000.00	
4. Texture Analyzer	01	600000.00	01	930000.00	
5. Colorimeter	01	200000.00	01	295000.00	
6. Die Set-Oceania mini tray sealer	01	500000.00	01	446470.00	
(c) Other capital items					

2. E ablishment/renovation facilities

Description of facilities	Newly established		Upgraded/refurbished		Remarks
	PP Target	Achievement	PP Target	Achievement	
NA	NA	NA	NA	NA	NA

3. Training/study tour/ seminar/workshop/conference organized

Description	Number of participants			Duration (Days/weeks/ months)	Remarks
	Male	Female	Total		
Coordination component (BARC)					
Inception Workshop (1 no)	56	7	63	1 day	All workshops held at the Conference room of BARC as per schedule of activity of the Coordination component
Half yearly Research Prog. Review Workshop (3 no.)	65+ 62+72	9+8+10	226	1+1+1 = 2 days	
Annual Research Prog. Review Workshop (2 no.)	65+63	9+8	1145	1+2 =3 days	
Project Completion Report Review Workshop (1 no)	52	6	58	1 day	

Orientation on PCR preparatory guideline	12	05	17	01 Day	All Principal Investigators of the sub project components attended the program
Component-1 (NSTU)					
(a) Training (Lakshmipur)	-	40	40	1	Household training on FP intervention
(b) Training (Noakhali)	-	40	40	1	
(c) Training (Khagrachari)	-	40	40	1	
(d) Workshop (Lakshmipur)	-	40	40	1	Workshop on FP consumption awareness
(e) Workshop (Noakhali)	-	40	40	1	
(f) Workshop (Khagrachari)	-	40	40	1	
Component-2 (RU)					
Product Launching workshop	50	15	65	1 day	Products demonstration

C.Financial and physical progress (combined & component wise)

Combined progress

Fig in Taka

Items of expenditure/activities	Total approved budget	Fund received	Actual expenditure	Balance/ unspent	Physical progress (%)	Reasons for deviation
a. Contractual staff salary	5684658	5684658	5684658	0	100	Not applicable
b. Field research/lab expenses and supplies	12681937	12665475	12665475	0	100	
c. Operating expenses	1078186	958165	958164.5	0	100	
d. Vehicle hire and fuel, oil & maintenance	915854	828566	828566	0	100	
e. Training/workshop/ seminar etc.	1019200	947800	947800	0	100	
f. Publications and printing	481000	477000	477000	0	100	
g. Miscellaneous	377565	375456	375456	0	100	
h. Capital expenses	5298600	5297450	5297450	0	100	
Total	**27537000**	**27234570**	**27234570**	**0**	**100**	

Coordination component (BARC)

Fig in Taka

Items of expenditure/activities	Total approved budget	Fund received	Actual expenditure	Balance/ unspent	Physical progress (%)	Reasons for deviation
a. Contractual staff salary	961167	961167	961167	0	100	Not applicable
b. Field research/lab expenses and supplies	481624	481624	481624	0	100	
c. Operating expenses	267523	152795	152795	0	100	
d. Vehicle hire and fuel, oil & maintenance	180359	95560	95560	0	100	
e. Training/workshop/ seminar etc.	397200	325800	325800	0	100	
f. Publications and printing	300000	297000	297000	0	100	
g. Miscellaneous	77127	76390	76390	0	100	
h. Capital expenses	398000	398000	398000	0	100	
Total	**3063000**	**2788336**	**2788336**	**0**	**100**	

Component-1 (NSTU)

Fig in Taka

Items of expenditure/activities	Total approved budget	Fund received	Actual expenditure	Balance/ unspent	Physical progress (%)	Reasons for deviation
a. Contractual staff salary	1955677	1955677	1955677	0	100	Not applicable
b. Field research/lab expenses and supplies	5652135	5650263	5650263	0	100	
c. Operating expenses	273535	271925	271925	0	100	
d. Vehicle hire and fuel, oil & maintenance	396900	396900	396900	0	100	
e. Training/workshop/ seminar etc.	480000	480000	480000	0	100	
f. Publications and printing	156000	155000	155000	0	100	
g. Miscellaneous	78753	78370	78370	0	100	
h. Capital expenses	1072000	1072000	1072000	0	100	
Total	**10065000**	**10060135**	**10060135**	**0**	**100**	

Component-2 (RU)

Fig in Tk

Items of expenditure/activities	Total approved budget	Fund received	Actual expenditure	Balance/ unspent	Physical progress (%)	Reasons for deviation
a. Contractual staff salary	2767814	2767814	2767814	0	100	Not applicable
b. Field research/lab expenses and supplies	6548178	6533588	6533588	0	100	
c. Operating expenses	537128	533445	533445	0	100	
d. Vehicle hire and fuel, oil & maintenance	338595	336106	336106	0	100	
e. Training/workshop/ seminar etc.	142000	142000	142000	0	100	
f. Publications and printing	25000	25000	25000	0	100	
g. Miscellaneous	221685	220696	220696	0	100	
h. Capital expenses	3828600	3827450	3827450	0	100	
Total	**14409000**	**14386099**	**14386099**	**0**	**100**	

D. Achievement of sub-project by objectives (Tangible form): Technology generated/developed

Component-1 (NSTU)

General/specific objectives of the sub-project	Major technical activities performed in respect of the set objectives	Output (i.e. product obtained, visible, measurable)	Outcome (short term effect of the research)
Component 1 To know the intra-household food consumption, food allocation, and nutritional outcomes of adolescent girls through the survey;	Conducted a baseline-end-line survey with training on fish powder consumption, consumer preferences, and collecting anthropometric data.	Understanding Food consumption patterns, nutritional intakes and conditions in adolescent girl's blood	Understanding the nutritional/anemic situation of adolescent girls will help in further developmental project
To develop Ready-to-use fish-based nutrition-dense products using the most suited fish species	Selecting species based on nutritional quality, affordability and availability and assess their quality, appearance and microbial load; nutrition analysis	13 types of Fish powder had been developed	Nutritious Fish powder will contribute in biomarkers increment in adolescent girl.
To conduct randomized controlled trials (RCTs) using Ready-to-use supplementary fish-	16-week intervention with Fish powder in daily food intakes, monitoring their activities, collecting blood serums.	Biomarkers increment in blood serum of adolescent girl by Fish powder fortification in daily food items.	Fish powder can enrich nutritional status of adolescent girl across country.

General/specific objectives of the sub-project	Major technical activities performed in respect of the set objectives	Output (i.e. product obtained, visible, measurable)	Outcome (short term effect of the research)
based products.			
To evaluate the shelf life of Fish powder preservation	Fish powder preservation treatment in two bangs, zipped bag and air tight bag, test continued up to 45 days	Shelf life was found for 1 year in room temperature.	Long shelf life can help in extensive supply and preservation of Fish powder countrywide.
To observe the consumer preferences for consuming the Fish powder	100 families with 401 members were interviewed about the test, color, smell and overall acceptances of Fish powder.	249 responses were found complete and valid for the analysis and more than 50% accepted FP warmly.	FP can act as a RFP countrywide among different group of people as nutritious food supplement.

Component-2 (RU)

General/specific objectives of the sub-project	Major technical activities performed in respect of the set objectives	Output (i.e. product obtained, visible, measurable)	Outcome (short term effect of the research)
To develop the Ready-to-cook (RTC) (Fish ball, Battered &breaded fish, fishsausage, Fish marinades,) and Ready-to-eat (RTE) (Fish crackers) from freshwater and marine fish;	All products have been developed by optimizing ingredient's amount and processing parameters Proximate composition, color and texture analysis were performed.	Developed 9 value added products; Nutritional quality of the products	Nine standard recipes are produced which can be used by small scale entrepreneurs
To determine the overall shelf-life of RTC and RTE products stored under modified atmosphere packaging at different storage temperature.	Shelf-life study of all products was completed by analyzing P^H, Free Fatty Acids (FFA), Total volatile base nitrogen (TVBN), Thiobarbituric acid reactive substances (TBARS), Aerobic plate count (APC) at different time interval.	Selected suitable gas mixture for each product to increase shelf life.	Established the shelf life of 9 products under MAP packaging

E. Information/knowledge generated/policy generated

Component-1 (NSTU)

General/specific objectives of the sub-project	Major technical activities performed in respect of the set objectives	Output	Outcome (short term effect of the research)
Food consumption pattern of adolescent girls	240 adolescent girls were interviewed by using 24-hours recall method.	Food component and its consumption amounts in 24 hours	Finding revealed lack of nutrition through food consumption of adolescent girl.
FP as a nutritious product for biomarker increment	Development of FP from five fish species, analyzed micronutrients and other nutritional values of FP	Enriched FP from whole fish with higher protein content	FP contribute as RFP in national nutrition index by increasing biomarkers.
Recipes development based on Fish powder	Appointing notionally expert chef for development of 7 different cooked recipes.	Delicate and delicious recipes were generated with its wide acceptances in daily food menu.	FP fortification and its acceptances can be accelerated by preparing different recipes.
Majority acceptance of fish powder in study areas	Distribution of FP among 100 families, conduction of surveys on smell, test and color of FP	35% people says that they are satisfied with the color, smell and taste of the product is good and 15% says that it is very good whereas 40% stays neutral.	Acceptance of FP can be an indication for further distribution countrywide.
Prolonged preservation period of fish powder	TVB-N values of fish powder were observed at 45°C and 55°C storage temperature after 15, 30 and 45 days	Shelf-life of fish powder was determined as 1 year by studying the TVB-N values and other parameter of fish powder at 55°C after 45 days later.	Long preservation period may instead the efficacy of FP as a potential nutritious product

Component-2 (RU)

General/specific objectives of the sub-project	Major technical activities performed in respect of the set objectives	Output	Outcome (short term effect of the research)
To know the consumer's acceptability of MAP packaged fishery products in the superstores of Dhaka city.	Survey method utilizing structured questionnaire	Consumer's acceptability of those 9 products was known	Feasibility of those 9 products in the market been revealed.

F. Materials development/publication made under the Sub-project

Component-1 (NSTU)

Publication	Number of publications		Remarks (e.g., paper title, name of journal, conference name, etc.)
	Under preparation	Completed and published	
Booklet/leaflet		02	I. Mamun, A. A, Sarwar S and Bhowmick (2020). Pushtimansomriddohchapila macher powder deyabivinnosushado khabar randhanpronali (in Bangla) Page 6; total number 650 II. Mamun, A. A, Sarwar S and Bhowmick (2022). Pustigunsomriddo Macher powder deya khabar toiri o khaddo o pustonirapottaeononnovhumika (in Bangla). Page 36 Total number 1200
Journal publication	03	01	1. Shuva Bhowmik, MUM Abu Zakaria, Md. Shahid Sarwar, SaymaBentaShofi, Syduzzaman, Farzana Akter, Md. Monirul Islam, Abdullah-Al Mamun, Development and nutritional index of ready to use fish products (RUFPs) from small fish species: Future superfoods for consumers, Applied Food Research, Volume 2, Issue 1, 2022, 100111, ISSN 2772-5022, https://doi.org/10.1016/j.afres.2022.100111. Abdullah-Al Mamun, Shuva Bhowmik, Md. Shahid Sarwar, SharminAkter, TanjinaPias, MUM Abu Zakaria, Md. Monirul Islam, Shakuntala HaraksinghThilsted and David C. Little Preparation and quality characterization of small pelagic fish powder: A novel ready-to-use nutritious food product for vulnerable populations" Under Review Measurement Food
Video clip/TV program		07	**Fish Powder Recipe Tutorial** 1.পুষ্টিকর মাছের পাউডার দিয়ে কচুরছড়ার মজাদার রেসিপি|| KocurChora with nutritious Fish Powder https://www.youtube.com/watch?v=FT4pwa-HXjg&list=PLhKs D8NnCB4plpj79opse00gIMEiyveze&index=1&t=8s 2.ফিশ পাউডারের মজাদার ও পুষ্টিকর ভর্তা || Mashed Fish Powder Recipe https://www.youtube.com/watch?v=ILuK8k1VJjg&list=PLhKsD8NnCB4plpj79opse00gIMEiyveze&index=2 3.ফিশ পাউডার দিয়ে বুটের ডাল রান্না || Buter Dal with Fish Powder Recipe https://www.youtube.com/watch?v=x1XQxuRkp5o&list

Publication	Number of publications		Remarks (e.g., paper title, name of journal, conference name, etc.)
	Under preparation	Completed and published	
			=PLhKsD8NnCB4plpj79opse00gIMEiyveze&index=3 4. ফিশপাউডার দিয়ে আলুর ডাল রান্না || Potato-Pulse curry with Fish Powder Recipe https://www.youtube.com/watch?v=kmMU01yLQnY&list=PLhKsD8NnCB4plpj79opse00gIMEiyveze&index=4 5.ফিস পাউডার দিয়ে কলমীশাক রান্না || Spinach curry with Fish Powder Recipe 6. মাছের কাটলেট এর সাথে ফিসপাউডার || Fish cutlet with Fish powder recipe https://www.youtube.com/watch?v=gOB2LQfOKmA&list=PLhKsD8NnCB4plpj79opse00gIMEiyveze&index=6 7.মাছের পাউডার দিয়ে আলু বেগুনের চচ্চড়ি || Potato-brinjal curry with Fish Powder Recipe https://www.youtube.com/watch?v=Udq9jgkakLk&list=PLhKsD8NnCB4plpj79opse00gIMEiyveze&index=7
News Paper/Popular Article		21	Fish Powder Project Activities published in different National TV channels and National and local newspapers on ***19.02. 2021*** (*Somoy tv News, Etv news, Jayjaydin news, Anandabazar, Tarun kantha, News24.com, Kalerkantha, Upakulbarta, Campustime 24, Mukta campus, Ankan, Barta bazar, Jagonews24.com, Business bangla, Alokito Bangladesh, Dhakatimes, DesjRupantar and Noakhali TV*)
Other publications, if any (Thesis)		8	1. Development and Nutritional Status of Ready to Use Punti (*Puntius ticto*) Fish Powder: A Super food for Consumers 2. Development and Nutritional Status of Ready to Use Anchovy (*Coiliadussumieri*) Fish Powder: A Super food for Consumers 3. Development and Nutritional Status of Ready to Use Sardine (*Salmophasia sardinella*) Fish Powder: A Super food for Consumers 4. Development and Nutritional Status of Ready to Use Kachki (*Coricasoborna*) Fish Powder: A Super food for Consumers 5. Development and Nutritional Status of Ready to Use Chapila (*Gudusiachapra*) Fish Powder: A Super food for Consumers 6. Shelf-life prediction of ready-to-cook Chapila (*Gudusiachapra*) fish powder storage at 45°C and

Publication	Number of publications		Remarks (e.g., paper title, name of journal, conference name, etc.)
	Under preparation	Completed and published	
			55°CConsumer preferences about about fish powder through intervention in daily food 7. Effect of fish supplementation to reduce inflammation in adolescent girls, A randomized controlled trial in three geographical locations in Bangladesh (CRP and Ferritin) 8. Effect of fish supplementation to mitigate anaemia in adolescent girls, A randomized controlled trial in three geographical locations in Bangladesh (Ferrous and Hemoglobin) 9. "Effect of fish supplementation on retinol and anthropometric indices in adolescent girls, A randomized trial control in three geographical locations in Bangladesh". 10. Effect of fish supplementation to mitigate vitamin B12 and Calcium deficiency in adolescent girls, A randomized controlled trial in three geographical locations in Bangladesh (vitamin B12 and calcium)
Component-2 (RU)			
Technology bulletin/ booklet/leaflet /flyer etc.	**1**	**1**	• Leaflet: রাজশাহী বিশ্ববিদ্যালয়ে গবেষণার মাধ্যমে প্রস্তুতকৃত এবং প্যাকেট জাতReady-to-Cook (RTC) & Ready-to-Eat (RTE) Fish Products (in Bengali) • Booklet on Technology Factsheet on Fish Product development and Packaging (under preparation)
Journal publication		**1**	• Development and quality evaluation of fish crackers under modified atmosphere packaging. 9th BFRF Fisheries Conference 2022 **(Receiver of best poster award)**
Video clip/TV program	**1**	**3**	• Detailed report captures on raw material collection to marketing of our products by Mati-o-Manush, BTV (to be broadcasted) • Detailed report on product preparation and packaging by Channel24 Krishi (13.06.22) • News on Research Finding workshop and product launching at RU by Channel24 (12.06.22) • News on Product launching workshop at BARC by Channel24 Krishi (16.03.22)
News Paper/Popular Article		**36**	• Daily Ittefaq (13.06.22) • Dhaka prokash24 (13.06.22)

Publication	Number of publications		Remarks (e.g., paper title, name of journal, conference name, etc.)
	Under preparation	Completed and published	
			• AmarNews (13.06.22) • Daily Kolomkotha (13.06.22) • Daily Upochar (12.06.22) • DainikDeshantor (12.06.22) • Dhumkatunews (13.06.22) • Bijoybangla news (12.06.22) • Daily ShomoyerAlo (12.06.22) • Dhakamail (13.06.22) • Amar Songbad (12.06.22) • Daily-bangladesh (13.06.22) • Bd-pratidin (13.06.22) • Amarrajshahi (13.06.22) • Bahannonews (12.06.22) • RidmikNews (12.06.22) • bbarta24.net (12.06.22) • Banglanew24 (12.06.22) • The dailycampus (12.06.22) • Kalerkantho (12.06.22) • Campuslive24 (12.06.22) • Mymensingheralo (14.06.22) • Rajtimes (12.06.22) • Sarabangla (12.06.22) • ProjonmoNews (12.06.22) • Silkcitynews (12.06.22) • Daily Amader Kantha (14.06.22) • Banglanews52 (13.06.22) • News24 (12.06.22) • Voice of new jersey (12.06.22) • Dhakareport24 (12.06.22) • Ajkerpatrika (13.06.22) • Rbcnews (15.06.22) • DainikAmadersomoy (12.06.22) • Daily Samakal (16.03.22) • Agrilife24 (18.03.22)
Other publications, if any		**1**	• Development and quality evaluation of tilapia crackers under modified atmosphere packaging (MS Thesis submitted 2022)

G. Description of generated technology/knowledge/policy

i. Technology factsheet

Component 1 (NSTU)

Technology factsheet 01

Title of the technology: ***Producing Fish powder from 5 different fishes***

Introduction

The dietary pattern of the world population has shifted mainly from staple foods to processed foods as a result of rapid urbanization, an increase in the number of working women, increasing disposable incomes and changing lifestyles (Sobol et al. 2018; Hu et al. 2019). Small sun-dried fish might be powdered and used to strengthen diets for infants, who are often deficient in micronutrients, to prevent this occurrence early on (Owino et al. 2008; Haug et al. 2010). Compared to dried fish, these Ready-to-use fish products (RUFPs) are less likely to produce an unpleasant fishy odor when added to prepare cuisine. Furthermore, RUFPs technology for food materials is booming and becoming popular (Fitzpatrick and Ahrné, 2005). RUFPs has a wide range of applications for which it is becoming increasingly popular (Islam et al., 2018; Monteiro et al., 2014). Furthermore, entire process (cleaning, drying, grinding) from raw fish to powder is economical, feasible and efficient (Modibbo et al., 2014). RUFPs is a rich source of nutrients and understanding its nutritional content is essential to balance its value as a supplementary food with other foods. Therefore, the present study aims to develop Fish powder from five indigenous fish species and evaluate its nutritional characteristics (proximate composition, minerals, fatty acid and amino acid) and potential contribution to the daily nutritional intake of infants, adults, and pregnant and lactating women.

Description

Fish samples were divided into three groups for the development of whole, gutted, and muscle RUFPs. For the development of powder from whole fish, samples were completely washed with water to eliminate dirt, dust, and other foreign matter (Fig 57). Descaling and eviscerating fish were followed by using a clean work table in hygienic conditions with the help of sterilized tools for the preparation of gutted fish powder. The fish fillet was separated from the Punti fish by using a sterile blade for the development of muscle powder. Then, whole fish, gutted fish, and muscle were subjected to sun drying for 5, 3, and 2 days, respectively, at 25–30°C. After drying, the whole fish, gutted fish, and muscle were ground by using an electric grinder into a fine powder (500 micron mesh) to obtain Punti Fish powder. The ground powder was packaged with high-density polyethylene and stored at 4ºC for nutritional analysis.

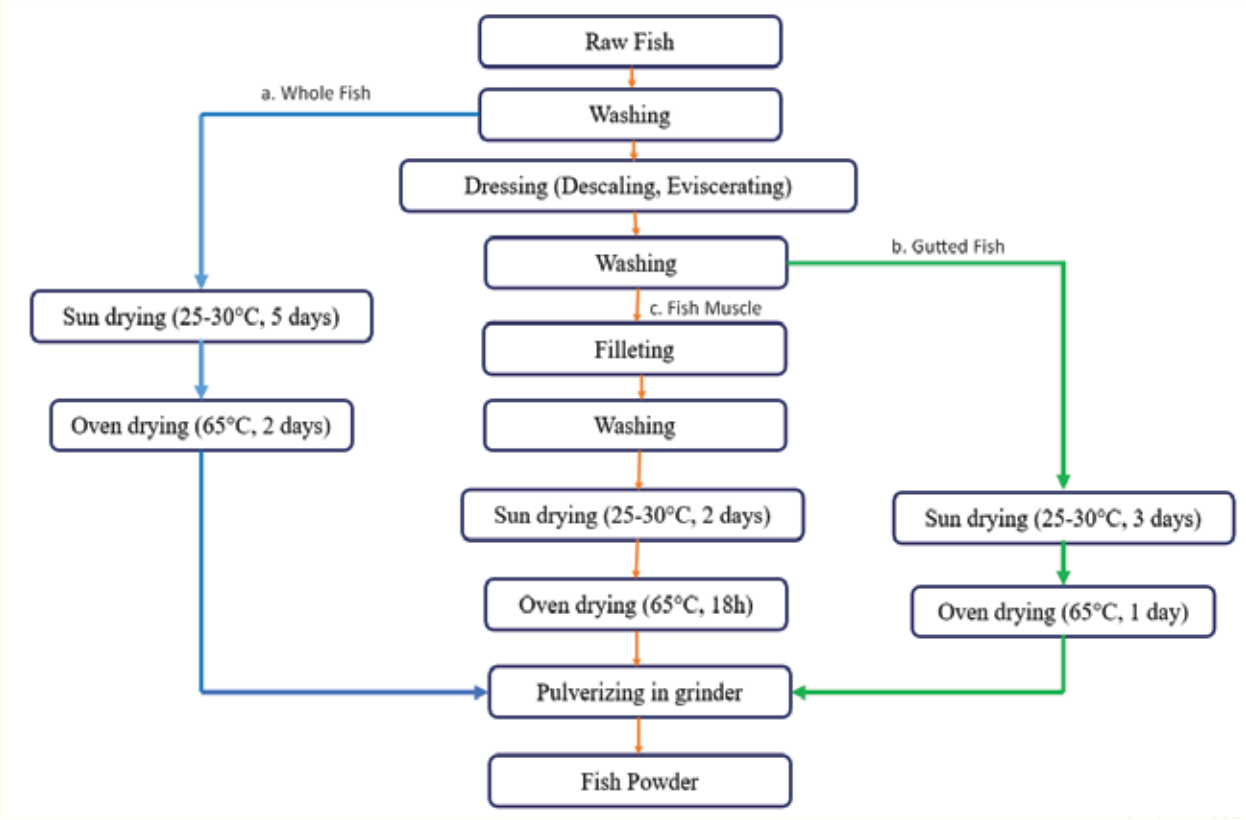


Fig 54. A schematic diagram of ready to use Fish powder (RUFP) preparation from whole, gutted and muscle portions of three freshwater fish species in Bangladesh

Suitable location/ecosystem

Normal room temperature and temperate zone is suitable for Fish powder production.

Benefits

Sun drying is the most viable and technologically potential way for the development of ready to use Fish powders (RUFPs) from small freshwater species. Study revealed that protein content of all RUFPs is very high and it could be a great ingredient for producing protein fortified food products. Additionally, RUFPs are also rich source of macro, micro and trace minerals. The concentrations of heavy metals were below the tolerable limits which indicate developed RUFPs is safe for human consumption. RUFPs are also high in essential and non-essential amino acid. Furthermore, RUFPs are great source of EPA, DHA, n-3 and n-6 fatty acid. The percentage of RNI results indicates that RUFPs will make a greater contribution to food and nutrition security for adults, infants, pregnant and lactating women. In addition, the developed RUFPs can be used as a novel raw material to develop health value-added products suitable for people of all ages and also play a key role to diminish hidden hunger.

Name and contact address of author

Abdullah-Al Mamun, PhD, Professor
Department of Fisheries and Marine Science
Noakhali Science and Technology University
Cell: +8801712928710
E-mail: mamun_au22@yahoo.com

- **Technology factsheet 02**
  Title of the technology: ***Development of 9 recipes from Fish powder***

Introduction

In Bangladesh, small indigenous fish species are precious and readily available source of protein, vitamin and micronutrients-rich food that is not typically found in other foods products (Hossain et al., 1999). The majority of micronutrients deficiencies in human and micronutrients supply from fish are found high concentrations in fish bones. Small fish that are usually eaten with organs and bones contain a lot of calcium and possibly iron and zinc (Musa, 2009). However, apart from eating small-sized fish species whole (with the bones inclusive), consumption of fish bones of larger fish is rarely practiced. Small indigenous fish need more time to clean rather than large fish which make middle and affluent class (MAC) people incurious to buy because they don't have plenty of time. Children who do not eat small fish are more prone to stunting and hidden hunger than children who eat small fish (Biesalski 2013; Marinda et al. 2018). On the other hand, it's very difficult for children to eat small fish with bones and organs. Furthermore, many children are accustomed to eating snacks such as biscuits, soup, and burgers and avoid raw fish in their regular diet due to an abundance of soft and Y-bones (Thorsdottir et al. 2009; Kasozi et al. 2018; Tiwali et al. 2020). In these circumstances, formulation of delicate and delicious recipes with Fish powder fortification will enhances the acceptances of fish and its powder worldwide.

Description

Professional chef was appointed to process some experimental recipes. Among them 9 were successfully accepted locally. Formulation of those recipes are described below:

Recipe 1: Mashed Fish powder
Ingredients:

→ 40 grams of fish powder
→ 3 large cloves of garlic
→ 4 dried chilies
→ The onion is a big one

Preparation method

At first, the fish powderwastaken in a heavy pan. Then garlic and dried chilieswere added. After cooling, one by one, everything was taken with the batter and if necessary, mixed with some salt, it will make delicious Fish powder mash.

Recipe 2: Boot pulses with Fish powder
Ingredients

→ Boot pulses 250 grams
→ 30 grams of fish powder
→ 2 onion sprouts
→ 1 teaspoon of garlic paste
→ Ginger mashed 1 teaspoon

→ 3 teaspoons of chili powder
→ 1 teaspoon of turmeric powder
→ Three tablespoons of oil
→ Cumin powder 1/2 teaspoon
→ 3 cardamoms, 1 cinnamon stick, 2 bay leaves
→ Coriander leaves

Preparationmethod

The boot stalks must first be cooked in a salt and turmeric solution. The onion must then be fried in oil in a skillet before hot spices like ginger, garlic paste, turmeric, chilli, cumin powder, and Fish powder are added after it turns a light golden color. If there isn't any water added, it will burn. Then it must be fried once more while adding the boiled boot stalks. Checking the salt is necessary. The broth must be served once it has decreased over time. You may add onion fries. It will be a better test.

Recipe 3: Potato pulses with Fish powder

Ingredients

→ 250 grams of potatoes
→ 30 grams of fish powder
→ 2 onion sprouts
→ 1 teaspoon of garlic paste
→ Mashed ginger 1 teaspoon
→ Tomato 1 cut into four pieces
→ 3 teaspoons of chili powder
→ 1 teaspoon of turmeric powder
→ Two tablespoons of oil
→ Cumin powder 1/2 teaspoon
→ 3 cardamoms, 1 cinnamon stick, 2 bay leaves
→ Coriander leaves

Preparation method

First, potatoes need to be boiled and need to break in half. Then, onions need to be fried in oil in a pan and after it becomes light golden color, hot spices, ginger, garlic paste, turmeric pepper and Fish powder need to be added and fried more. Little water needs to be added for preventing burning. Then, it needs to fry again with the boiled potatoes. When it is completely dry, it needs to shake well with 2 cups of water and tomato pieces and cover it. After a while, it needs to serve with cumin powder and coriander leaves and serve with sweet Fish powder and potato dal.

Recipe 4: Kalmispinach with Fish powder
Ingredients

→ Two handfuls of grafted vegetables
→ 40 grams of fish powder
→ 100 grams of lentils
→ 2 onion sprouts
→ 1 teaspoon of garlic paste
→ 3 teaspoons of chili powder
→ 1 teaspoon of turmeric powder
→ Two tablespoons of oil
→ The amount of salt

Preparation method

First, the onion needs to fried in oil in a pan and after it becomes light golden color, garlic paste, turmeric pepper, Fish powder and salt need to be added. Little water needs to be added for preventing burning. Then, it needs to fry again with lentils. After that, the grafted vegetables need to be picked and washed. Pan needs to cover with a lid, so that, water will come out from the grafted vegetables. It needs to mixed for a while to reduce the broth and with a little water it needs to cover again. The flame will be moderate. I will check the salt, if necessary. It needs to be dried, roasted and served with Fish powder.

Recipe 5: Pumpkin soup with Fish powder
Ingredients

→ 250 grams of pumpkin
→ 3 tablespoons of oil
→ 30 grams of fish powder
→ 2 onion sprouts
→ 1 teaspoon of garlic paste
→ 1 teaspoon of chili powder
→ 1 teaspoon of turmeric powder
→ The amount of salt
→ The amount of water
→ 1 tablespoon coriander leaves

Preparation method

First fry the onion in oil in a pan and after it becomes light golden color, I fry it well with garlic paste, turmeric pepper, Fish powder and salt. Give a little water. Then you have to roast it for a while with the chopped rice. After a while the broth decreases and you have to cover it again with three cups of water. The flame will be moderate. I will check the salt, if necessary. If the broth is reduced, you have to check whether it is boiled or not, otherwise more water will be required. When it is well cooked, the broth will reduce a little and served it with coriander leaves.

Recipe 6: Potato pulse curry with Fish powder
Ingredients

→ 200 grams of lentils
→ Potatoes cut into cubes 100 g
→ 3 tablespoons of oil
→ 30 grams of fish powder
→ 2 onion sprouts
→ 1 teaspoon of garlic paste
→ 3 teaspoons of chili powder
→ 1 teaspoon of turmeric powder
→ The amount of salt
→ The amount of water
→ 1 tablespoon coriander leaves

Preparation

The onion must first be fried in oil in a skillet, and once it turns a light golden color, salt, turmeric pepper, and Fish powder must be added and further fried. After addition, a little water will stop the burning. It must then be fried once more while adding lentils. The potatoes must then be roast for a bit. The broth will eventually diminish, and you'll need to add another half cup of water to cover it. There won't be much flame. If necessary, salts must be supplied as tests. When the oil is truly roasted on top, coriander leaves must be served with it.

Recipe 7: Taro root with Fish powder
Ingredients:

→ 500 grams of water hyacinth
→ Fish powder 2 teaspoons
→ Two onions
→ Half a teaspoon of turmeric powder
→ 1 teaspoon of chili powder
→ Half a teaspoon of coriander powder
→ Half a teaspoon of cumin
→ 1 teaspoon of garlic paste
→ Ginger pastes half a teaspoon
→ Cardamom makes two cinnamon bay leaves
→ Four to five slices of green chilies
→ The amount of oil
→ Salt to taste
→ According to the amount of coriander leaves

Preparation method:

First, taro roots need to boil the face and remove the peel. Half of it will be well mashed and the other half will be halved. Heat oil in a pan, lightly fry onion pieces in it, add whole cumin,

cardamom, cinnamon, bay leaves, stir for a while and add remaining spices and salt. Now add some water and add Fish powder. Now cook on medium heat for four to five minutes. Now you have to give Taro root. Shake the face well and give it hot water and mix it. Cook for 5 to 7 minutes. When it boils, put sliced green chilies in it with coriander leaves and leave it for 1 minute.

Recipe 8: Fish cutlet with Fish powder
Ingredients:

→ Two boiled potatoes
→ Boiled raw banana a
→ Beresta 1 tsp
→ 1 teaspoon of salt
→ Three young chilies
→ 1 teaspoon of coriander powder
→ One egg
→ 1 cup of biscuit powder
→ 4 tablespoons of fish powder

Preparation method:
Potatoes need to be boiled. Then boil raw bananas and the Fish powder, salt, raw chili, coriander powder needs to mix with it and cut it. Then lightly fried to make a fun fish cutlet.

Recipe 9: Potato eggplant chutney with Fish powder
Ingredients:

→ Light 250 g large two
→ Two large eggplants
→ 30 g of powder 3 tsp.
→ 2 tablespoons chopped onion
→ Spoon of crushed garlic
→ Like the amount of salt
→ 1 teaspoon of turmeric powder
→ 3 tablespoons of oil
→ 1 teaspoon of cumin powder
→ 3-4 green chilies
→ The amount of water

Preparation method:
Potatoes and eggplant should be cut into cubes. In a pan fry onion with oil till it becomes light brown then stir in garlic and chilly. Mix turmeric powder with a little water, cumin powder and Fish powder. Then shake it with potatoes and eggplant and cover it with adequate amount of salt and water. When the broth is reduced to semi dry condition, it should be taken down and served.

Suitable location/ecosystem:

Daily food for consumers in each HH of the country is suitable for fish fortification by those recipes.

Benefits

Compared to dried fish, these Ready-to-use Fish products (RUFPs) are less likely to produce an unpleasant fishy odor when added to prepare cuisine. Furthermore, RUFPs technology for food materials is booming and becoming popular (Fitzpatrick and Ahrné, 2005). RUFPs has a wide range of applications for which it is becoming increasingly popular (Islam et al., 2018; Monteiro et al., 2014). Furthermore, entire process (cleaning, drying, grinding) from raw fish to powder is economical, feasible and efficient (Modibbo et al., 2014). RUFPs are a rich source of nutrients and understanding its nutritional content is essential to balance its value as a supplementary food with other foods.

Name and contact address of author
Abdullah-Al Mamun, PhD, Professor
Department of Fisheries and Marine Science
Noakhali Science and Technology University
Cell: +8801712928710
E-mail: mamun_au22@yahoo.com

ii. Component-2 (RU)
Technology factsheet 01

Title of the technology: ***Development of Ready-to-cook (RTC) Fish ball***

Introduction: Fish balls are rounded meat balls made from fish paste which are then boiled or deep fried. Similar in composition to Fishcake, Fish balls are often made from fish mince or surimi, salt, and a culinary binder such as tapioca flour, corn, or potato starch. Fish balls are popular in East and Southeast Asia, where it is eaten as a snack or added to soups or hotpot dishes. They are usually attributed to Chinese cuisine and the Fish ball industry is largely operated by people of Chinese descent. European versions tend to be less processed, sometimes using milk or potatoes for binding. Nordic countries also have their own variation. Fish ball is also popular snacks items in Bangladesh. Fish ball are not much available in the market compared to that of chicken or other meat ball. However, there are different preparation process are practiced. Therefore, a standard recipe is required to make this product easily available and consumable. Small scale entrepreneurs can easily prepare the Fish ball using the recipe and start a ssustainable business.

Description: Fish ball was prepared by the following optimized standard recipe.

Ingredients required*

- Corn flour- 40 g
- Ginger paste-10 g
- Garlic paste- 8 g

- Corriander-2.5g
- Cumin-2.5g
- Red chili powder- 3 g
- Black Pepper powder-1.5 g
- Soy sauce- ½ tsp
- Lemon juice- 2 tsp
- Salt- 7.5 g
- Onion-40g
- Green chili-4 pcs
- Bread crumbs- ½ cup

Breaded ingredients**

- Egg-2
- Bread crumbs- ½ cup

At first the fishes were washed, beheaded and eviscerated. The skinned fishes were filleted and deboned manually under chilled condition. The minces were prepared by a mechanical meat mincer (Panasonic Corporation, Japan). For the preparation of Fish ball, 500 g of fish mince was taken in a bowl and then added different ingredients* and mixed well. The mixture was then kept at refrigerator for 30 min. Then fish minces were converted to round shaped ball manually. The prepared ball was then soaked in beaten eggs and rolled on bread crumbs**. Fish ball was then ready for MAP packaging and preservation at 4°C.

Suitable location/ecosystem: Any location where low-cost fish is available.

Benefits

- Easy preparation
- Less instrument involvement
- Suitable for small scale business

Name and contact address of author

Dr. Md. Tariqul Islam, Professor,
Department of Fisheries,
University of Rajshahi, Rajshahi.
Phone: +88-01712697251,
Email: tariqrubd@gmail.com

Technology factsheet 02

Title of the technology: ***Development of Ready-to-cook (RTC) Fish sausage***

Introduction: KeropokLekor is kind of Fish sausage very popular in some parts of Malaysia. It is a popular snack originated from the state of Terengganu in Malaysia, home to the fishing industry. Fish paste are normally ground together with sago flour and seasoned with salt and

sugar. The contents are then mixed together, kneaded and formed into long, sausage-like shapes. There are different ways to enjoy KeropokLekor. It can be fried in its rolled form and the result will be chewy. It can be thinly sliced and fried, and it will be crispy like chips. It can also be boiled or steamed so it will be soft like eating Fish balls. All of these types actually taste slightly different from each other, although they are made from the exact same ingredients. Also, they can be eaten just like that or dipped in chili sauce.

Description:
KeropokLekor type Fish sausage was prepared by fish mince with adding different ingredients and spices.

Ingredients required*

- Corn flour-160 g
- Wheat flour-80g
- Sugar-14g
- Salt- 11g
- Ice-60-100g
- Pepper- 2g
- Chilli powder- 1.5g
- Soya sauce-1/2 tsp

Fish (Pangas/Mackerel) mince was prepared similar to that of Fish ball preparation.Firstly, 500 g of fish mince was taken in a bowl and then added different ingredients* and mixed well. The mixture was entered into the sausage stuffer to prepare Fish sausage. Then thecylinder-shapedsausage was boiled at hot water until floated. Fish sausage was then ready for MAP packaging and preservation at 4°C.

Suitable location/ecosystem: Any location where low-cost fish is available.

Benefits

- Easy preparation
- Less instrument involvement
- Suitable for small scale business

Name and contact address of author
Dr. Md. Tariqul Islam, Professor,
Department of Fisheries,
University of Rajshahi, Rajshahi.
Phone: +88-01712697251,
Email: tariqrubd@gmail.com

Technology factsheet 03

Title of the technology: ***Development of Ready-to-cook (RTC) Battered &breaded Fishfillet***

Introduction: Batter and breading are actually one kind of coating technology. Batter is the liquid preparation from ground cereals, spices, salt, sugar and other ingredients and/or additives for coating. Typical batter types are: non-leavened batter and leavened batter. On the other hand, breading is the dry breadcrumbs or other dry preparations mainly from cereals with colorants and other ingredients used for the final coating of fishery products. Typical breading types are: free-flowing breading, coarse breading, and flour-type breading. Usually battered products are breaded for getting good quality products. There are a variety of Batter and breading formulations. The flour and starch level are usually higher in batter mixes, which account for 80 to 90 percent of the total. In breading mixes, flour and starch usually account for 70 to 80 percent, with salt and other seasonings making up the remainder. Batters and breading enhance product appearance (for example, colour), texture, and flavour. They also act as a moisture barrier, holding in natural juices, thus often making the product more tender. Different kinds of Battered and breaded fishery products are produced from fishes, mollusks and crustaceans. Among them, battered Fish finger and Fish fillets are very common in the market. The demand of this kind of products is increasing day by day.

Description:

Ingredients required*

- Ginger paste-10 g
- Garlic paste- 10 g
- Cumin powder- 2 g
- Coriander powder-2 g
- Red chili powder- 4 g
- Turmeric powder-2 g
- Soy sauce- ½ tbs
- Lemon juice- 2 tsp
- Salt- 7 g

Breaded ingredients**

- Wheat flour- 1 cup with Cumin 2 g, Red chili- 2 g, Salt
- Egg-2
- Bread crumbs-1 cup

Battered & breaded fish was prepared from the fish fillets. Fishes (Tilapia/white Snapper) were filleted first and kept aside. Different ingredients* were mixed well in a bowl. The mixture of ingredients was rubbed well on the fillets and kept at the refrigerator for 1h. After that, the fillets were rolled on flour-spices mixture**, soaked in beaten eggs and finally rolled on breadcrumbs. Then the products were kept at refrigerator for 30 min with plastic wrap. Then the Battered and breaded Fish fillets were ready for MAP packaging and preservation at 4°C.

Suitable location/ecosystem: Any location where low-cost fish s available.

Benefits:
- Easy preparation
- Less instrument involvement
- Suitable for small scale business

Name and contact address of author
Dr. Md. Tariqul Islam, Professor,
Department of Fisheries,
University of Rajshahi, Rajshahi.
Phone: +88-01712697251,
Email: tariqrubd@gmail.com

Technology factsheet 04

Title of the technology: ***Development of Ready-to-cook (RTC) Fish marinades***

Introduction: Marinades are, by general definition, fish or fish portions processed by treatment with edible acids and salt, and put up in brine, sauce or oil. Adjuvants for seasoning and bleaching are also often used. Fish marinades are characterized by the typical marinated odour and flavour. Treatment with acetic acid and salt brings about a sort of refinement in the fish simultaneously developing the typical marinated odour and flavour. Further improvement in odour and flavour is brought about by addition of various spices and covering liquids. Pelagic fatty fish such as Sardine, Herring etc. are generally used to process marinades, though marinades are also processed out of several other species of fish and shellfish. In order that the characteristic odour, flavour and taste of marinades are maintained, use of additives which can suppress spoilage and prolong shelf life is restricted. Therefore, marinades have only a limited shelf life and are classified among the semi-conserves.

Description

Soaking Solution*
- Acetic acid-2%
- Salt-10%

Others Ingredients**
- Garlic-7.5g
- Ginger-5 g
- Red chili-2.5 g
- Coriander-1.5 g
- Turmeric- 1.5g and
- Cumin-1 g

Sardine fishes were cut into slices and washed with water. First 500 g of fish slices was taken in a bowl and added 10% salt and 2% acetic acid solutions. Then stored at refrigerator (4°C) for 48 h. The fish slices were taken out and drained off the solution. Then different ingredients ** were

added and mixed well with the fish slices. Then Fish marinades were ready for MAP packaging and preservation at 4°C

Suitable location/ecosystem: Any location where low-cost fish is available.

Benefits

- Easy preparation
- Less instrument involvement
- Suitable for small scale business

Name and contact address of author
Dr. Md. Tariqul Islam, Professor,
Department of Fisheries,
University of Rajshahi, Rajshahi.
Phone: +88-01712697251,
Email: tariqrubd@gmail.com

Technology factsheet 05

Title of the technology: ***Development of Ready-to-eat (RTE) Fish crackers***

Introduction

Many Southeast Asian countries consider Fish crackers to be exotic snack foods. The Fish crackers can be a popular Ready-to-eat snacks item for all cluster of people. It can be a great choice to replace the potato crackers which is mainly contain carbohydrates, but Fish crackers contain protein along with carbohydrates. Beside the fish flesh as the protein source, starch is a crucial ingredient in making Fish crackers. Starch is used to impart viscidness and consistency of the crackers. Various starch flour such as tapioca, wheat, corn, sago, rice and even mug bean is available for Fish cracker manufacture. Good quality cracker must have adequate expansion on puffing for crispness, as well as low moisture content and low oil absorption. A number of previous studies have attempted to develop the quality and appropriateness of Fish cracker.

Fish at large are distributed into two main clusters which are marine and freshwater fishes. In the Fish cracker production, marine fishes are usually been used by many manufacturers since most of them live in the coastal area. Some of the marine fish supposedly used for Fish cracker preparation are Sardine (*Clupea leiogaster)*, Jewfish (*Johnius soldado*), Big-eye (*Brachydeuterusauritus*), Snapper (Lutjanus spp), Yellow pike conger (*Ophiocephalusmicropeltis*), and Featherback (*Natopteruschilata*). Some viable Fish crackers in Malaysia are found to have used Yellow-banded trevally (*Selaroidesleptoleptis*), Wolf herring (*Chirocentrus dorab*) and Round herring (*Dussumieriahasselti*) as the fish source. In Indonesia, Barredspanish mackerel (*Scomberomeruscommersoni*) is used as the fish source. The profitable Fish crackers in Thailand are usually produced from Tuna, Sardine and Mackerel, though the choice of fish depends on accessibility, cost and the required quality of the final product. However, with the progress of aquaculture technology, some cultivated fresh water fish can be used as the raw material for Fish cracker manufacture. Various freshwater fishes are being

cultured through this technique such as Dory, Carp, Catfish (Pangasius spp.), and many more. Bighead carp (*Hypophthalmichthys nobilis*), Dory (*Pangasius hypothalamus*) and Rohu (*Labeorohita*) are the freshwater fishes those are believed has the probable to be used in the Fish cracker industry. The production of minced Fish product is a well-established practice that allows rational use of fishery by-products and low-value fish species.

Description

Ingredients*

- Tapioca flour-100 g
- Sugar-6 g
- Salt-5.5 g
- Ice-15 g, and
- Sodium bicarbonate-1 g

Fish (Tilapia/Tuna) mince was prepared similar to that of Fish ball preparation. First 90g of fish mince was taken in a bowl and added ingredients* and mixed well. The dough was shaped into cylindrical and steamed for 30 m followed by chilling in refrigerator overnight. On the next day, cylinder shaped product was cut into 2 mm thickness and put into oven of 50°C for 10 to 12 hours or sundried for a day until the moisture content reading was 10% ± 2%. Dried crackers were then deep fried in vegetable oil at 170-180°C. Then the Fish crackers were ready as Ready-to-eat (RTE) products for MAP packaging and preservation at room temperature.

Suitable location/ecosystem: Any location where low-cost fish is available.

Benefits

- Easy preparation
- Less instrument involvement
- Suitable for small scale business

Name and contact address of author

Dr. Md. Tariqul Islam, Professor,
Department of Fisheries,
University of Rajshahi, Rajshahi.
Phone: +88-01712697251,
Email: tariqrubd@gmail.com

Technology factsheet 06

Title of the technology: ***Modified atmosphere packaging of RTC and RTE fish products***

Introduction

Modified atmosphere packaging (MAP) is a packaging technology where the air inside the package is replaced by an exact gas or a mixture of gases that differ from the air composition. MAP packaging combined with low storage temperatures is an effective technique to achieve

shelf-life extension of fishery products (Sivertsvik*et al*. 2002). MAP is a widely used packaging technique for displaying chilled fish, meat and their products in developed countries. It extends the shelf-life (25-400%) of the raw fish fillets at refrigeration temperature (Reddy *et al*. 1991; Pantaza*et al*. 2008). It can also increase the shelf-life under room temperature. It allows retailers to sell raw fishes and their products for an extended period. In our previous study, the highest shelf life of sliced Tilapia was 15 days for MAP 1 (50%CO_2 & 50%N_2) followed by 12 days for MAP 2 (50% CO_2 & 50% O_2), 9-12 days for vacuum pack and 6-9 days for no pack (control) sample (Karim et al. 2020). In case Rohu fish, the highest shelf life was 16 days for MAP 1 (50%CO_2 & 50%N_2) followed by 13 days for MAP 2 (50% CO_2 & 50% O_2), 11 days for vacuum pack and 8 days for no pack (control) sample (Das et al. 2021). On the other hand, the highest shelf life of sliced Goonch fish (Baghair) was 12 days for MAP 1 (50%CO_2 & 50%N_2) followed by 10 days for vacuum pack, 9 days for MAP 2 (50% CO_2 & 50% O_2), and 6 days for no pack (control) sample (Alice et al. 2020). However, vacuum packaging significantly increased the shelf-life, but it is not suitable for all types of fishery products particularly for soft and breakable products, as the pressure created during vacuum packaging can break down the products structure inside the pack. Therefore, MAP packaging with thermoformed tray (using Tray Sealer) instead of pouch is more convenient for displaying the products for the consumer. However, very study was found to increase the shelf life of those value-added products by combination of MAP packaging and refrigerated storage condition. Therefore, there is a scope to produce different value-added products from low value fish and preserve those fishery products under MAP packaging condition in different storage condition to get the sufficient shelf life for marketing in the superstores. In Bangladesh, this type of packaging system has not developed yet in the market. Therefore, development of different value-added products either RTC or RTE and preservation through MAP packaging along with refrigerated storage or room temperature is required to ensure the supply of quality fishery products with the extended shelf life in the market.

Description

The RTC fish products (Fish ball, Battered & breaded fish, Fish marinades and Fish sausage) were packed in tray (PP/EVOH/PP; 760 µm) with plastic top (PE/PA/PE; 100 µm). Four types of packaging were applied under modified atmosphere packaging with different gas ratio using the method described by Noseda et al. (2012) with some modifications using a tray sealer (Oceania mini, Italian Pack, Italy) attached with Gas Mixer KM100-3 MEM (WITT, Germany). Analysis of the O_2, N_2 and CO_2 levels in the headspace of the packaged samples were performed with Oxybaby M+ gas analyzer (WITT, Germany). Those four types of packaging were used as treatments namely, (a) aerobic, not sealed as control; (b) MAP 1 (50%CO_2 & 50%N_2) as treatment-1; (c) MAP 2 (40% CO_2, 30% N_2 & 30% O_2) at treatment-2 and (d) MAP 3 (50% CO_2 & 50% O_2) as treatment-3. On the other hand, RTE fish products were packed in plastic film (PE/PA/PE; 100 µm) performed in MAP packaging machine (C100, Multivac, Germany). Three types of packaging were used as treatments namely, (a) air pack as control; (b) MAP 1 (100% N_2) as treatment-1; and (c) MAP 2 (50% CO_2 & 50%N_2) as treatment-2. The RTC and RTE products were stored at 4˚C and room temperature, respectively. The packaged products in

triplicate were subjected to biochemical and microbiological analysis (P^H, Free Fatty Acids (FFA), Total volatile base nitrogen (TVBN), Thiobarbituric acid reactive substances (TBARS), Aerobic plate count (APC)) in 4-10 days interval for RTC and a month interval for RTE products in the laboratory. One-way ANOVA with the application of Tukey test using SPSS Version 20 at $P < 0.05$ level of significance.

In this study, the highest shelf life of Tilapia and Tuna Fish ball were 20 and 16 days, respectively under MAP-3 (50% CO_2 & 50% O_2). MAP-1 (50% CO_2 & 50% N_2) demonstrated the highest shelf life of 26 days for both Pangas and Mackerel Fish sausage. MAP-2 (40%CO_2, 30% N_2 & 30% O_2) gave the highest shelf life of 16 days for both Battered &breaded Tilapia and white Snapper. Fish marinades showed the 34 days shelf-life under MAP-2 (40%CO_2, 30% N_2 & 30% O_2). Tilapia and Tuna Fish crackers demonstrated the highest shelf life of 5 and 6 months, respectively under both MAP-1 (100% N_2) and MAP-2 (50% CO_2 & 50% N_2). Therefore, these MAP packaging can be used to increase shelf-life of RTC and RTE fish products.

Suitable location/ecosystem: Anywhere having packaging machine, possibly insuperstores or large-scale fish processing industry.

Benefit

- Easily displayable
- Easily stackable
- Lucrative packaging
- RTC products can keep refrigerated condition
- RTE products can keep room temperature
- Suitable for large scale business

Name and contact address of author
Dr. Md. Tariqul Islam, Professor,
Department of Fisheries,
University of Rajshahi, Rajshahi.
Phone: +88-01712697251,
Email: tariqrubd@gmail.com

ii. Effectiveness in policy support

Component 1 (NSTU)

- Fish powder fortification can enrich the nutrition intakes of adolescent girls as well as others countrywide.
- FP can introduce entrepreneurs with profit from both economically and nutritionally.
- National nutritional goals can be proceed fasted by FP fortifications in daily food
- Developed recipes can be used in restaurants for raising awareness and conforming enough nutrition for daily consumers.

Component 2 (RU)

- MAP packaged value-added fish products can be introduced in the market based on the consumer's acceptability of those value-added products in three different outlets.

H. Technology/knowledge generation/policy support

i. Immediate impact on generated technology (commodity & non-commodity)

Component 1

- Fish powders have prolonged preservation time and high nutritional status. It also contributed in nutritional index of adolescent girl by increasing biomarkers in blood serum.

Component 2

- As five standard recipes of value-added fishery products has been developed, these can be utilized by anyone including small scale entrepreneurs in the country.
- Large scale entrepreneurs can think to utilize the MAP packaging technology to increase shelf-life of value-added fishery products

ii. Generation of new knowledge that help in developing more technology in future

Component 1

- Biomarkers, especially vitamin B12 increment in the body of adolescent girl of the country can be contributed by Fish powder fortification.

Component 2

- Technology generated under the present study will help to generate more advance technologies in the following areas-
- More value-added product development
- Improvement of MAP packaging technology to increase more shelf life.
- Combination of MAP technology with other technologies like using Nano-materials, cold plasma etc. to increase shelf life.

iii. Technology transferred that help increased agricultural productivity and farmers' income

Component 1

- Fish powder production line and marketing will create new entrepreneurs for circulation this nutritious food. It will benefit both farmers and consumers economically and nutritionally.

Component 2

Six technologies have already been proposed to NATP-2 PIU-BARC, out of which five are the development of five value-added fishery products such as Fish ball, Fish sausage, Battered & breaded fish, Fish marinades and Fish crackers and another one is Modified atmosphere packaging of RTC and RTE fish products. First five technologies have required less investment and last one has required large-scale investment. Out of six technologies, one technology that is preparation of fish crackers has already been taken

for validation at post-harvest technology division of Bangladesh Agricultural Research Institute (BARI), Gazipur. The validation process has already been partially completed. The trainees of the validation process were already started producing Ready-to-fry fish crackers and sell in different outlets around Dhaka city. Other technologies can also be disseminated by training and demonstration to the stakeholders. For the last technology (MAP packaging), large scale processor or superstores can come forward to establish a MAP packaging-based industry in the country. These technologies can help to supply the nutritious RTC and RTE value-added fishery products under MAP packaging in the country which can ultimately ensure convenient and lucrative packaged products in the market.

iv. Policy support

Component 1

- Contribution of Fish powder should be intervened countrywide as food supplement as well as flavored additive. As a RFP, it has already proved its efficacy in study areas. More concentration and awareness should be drawn on its production, distribution and feedbacks for future developments.

Component 2

- Consumer's acceptability of the fish products (under the study)has already been evaluated by survey method utilizing a structured questionnaire. As a result, policy maker can take step based on the survey results to introduce these kinds of value-added products in market which ultimately increase the value, convenience and price of the low-cost fishes. By this way, fish producers will be benefited through diversification of utilization of low value fishes in the country.

I. Information regarding desk and field monitoring

i. Desk monitoring

Consulting meetings, workshops, seminars, coordination meetings etc.	Date & Venue	Output
Component 1 (NSTU)		
1) Inception workshop	22/12/2019; BARC, Dhaka	Improvement of the sub project document
2) 1st& 2nd half yearly progress report	20.06.20 and 29.07.21 BARC; DHAKA	-Baseline activity of the sub project components initiated and information generated; -Procurement activity initiated
3) 3rd half yearly progress report	02.06.2022; BARC	Development of PCR draft done properly

Consulting meetings, workshops, seminars, coordination meetings etc.	Date & Venue	Output
4) 1st& 2ndCo-ordination meeting	07/09/2020; BARC, Dhaka	Necessary initiative taken and significant progress achieved within the reporting period
5) 1st Annual progress workshop	30/09/2020; BARC, Dhaka	Additional technical information improved the quality of research
6) Meeting with World bank representative	25/08/2021; BARC, Dhaka	Sub-project revision along with activity time plan
7) 2nd Annual progress workshop	23/11/2021; BARC Dhaka	Causes of difference in hilsa catch ban period in Bangladesh, India and Myanmar, discarded from the study
8) 3rd Co-ordination meeting	12/12/21; BARC Dhaka	-Nutrition rich fish products developed. -Linkage with entrepreneurs initiated
9) Progress meeting on zoom platform	02.05.2021, Virtual platform	Incorporated all review suggestions of PIU-NATP
10) 4th Co-ordination meeting	08.10.2022 BARC, Dhaka	PCR workshop implementation plan finalized
11)Workshop on PCR preparation	11/05/22; BARC Dhaka	Orientation guidelines improves the knowledge of PCR writing
12)Progress meeting on zoom platform	02.05.2021, Virtual platform	Incorporated all review suggestions of PIU-NATP
13)Draft PCR review workshop	25.10.2022	Adaption of recommendations of the workshop helped to improved the PCR draft
Component 2 (RU)		
1) Inception workshop	22/12/2019; BARC, Dhaka	Modification and improvement of project document
2) 1st& 2nd half yearly progress report	20.06.20 and 29.07.21 BARC; DHAKA	-Baseline activity of the sub project components initiated and information generated; -Procurement activity initiated
3) 3rd half yearly progress report	02.06.2022; BARC	Draft PCR developed as standard format of the PIU with required information
4) 1st& 2ndCo-ordination meeting	07/09/2020; BARC, Dhaka	Procurement of lab equipment's initiated
5) 1st Annual progress workshop	30/09/2020; BARC, Dhaka	Diversified fish species included and product quality maintained
6) Meeting with World bank	25/08/2021;	Sub-project revision along with activity time

Consulting meetings, workshops, seminars, coordination meetings etc.	Date & Venue	Output
representative	BARC, Dhaka	plan
7) 2nd Annual progress workshop	23/11/2021; BARC Dhaka	Improved fish product quality and shelf-life of products
8) 3rd Co-ordination meeting	12/12/21; BARC Dhaka	Procurement of all pending equipment's and chemicals completed as per revised plan
9) Progress meeting on zoom platform	02.05.2021, Virtual platform	Incorporated all review suggestions of PIU-NATP
10) 4th Co-ordination meeting	08.10.2022 BARC, Dhaka	PCR workshop implementation plan finalized
11)Workshop on PCR preparation	11/05/22; BARC Dhaka	Orientation guidelines improves the knowledge of PCR writing
12)Progress meeting on zoom platform	02.05.2021, Virtual platform	Incorporated all review suggestions of PIU-NATP
13)Draft PCR review workshop	25.10.2022	Adaption of recommendations of the workshop helped to improved the PCR draft

Plate 33. Sub project workshops and seminars

ii. Field monitoring

Date	No. of visit	Name/s of visitors	Addresses	Output
Component I (NSTU)				
19.02.20	1	Dr. Md. Monirul Islam	Member Director, (Fisheries), and Sub project Coordinator (BARC)	Visited Pharmacy lab, NSTU and put technical advice for further improvement
12 .09.20	1	Prof Didar-ul-Alam	VC of NSTU Sonapur, Noakhali	Encourages sub project activities and scientists inspired work
20.09.20	1	Prof. Dr. Mohammed Faruque Uddin	Treasurer of NSTU Sonapur, Noakhali	Cordially recommended the activity of project
07.10.20	1	1. Md. Abdur Rahman 2. Dipok Kumar 3. Md. Hasan Mahmud	Monitoring associates& Capacity dev. Associate, PIU, NATP. BARC	Visited research field and instruction given by the team was executed.
20.01.22	1	Dr. Md. Monirul Islam	Member Director, (Fisheries), and Sub project Coordinator (BARC	Visited research site. Advice given by Coordinator was executed.
20.01.22	1	Dr. Md. Harunur Rashid	Director, PIU-BARC, NATP-2	Visited research field; Some advice given; action was taken accordingly.
30.01.22	1	Dr. Saleh Uddin Ahammed	Consultant PBRG sub projects (Fisheries) BARC	Suggested shelf-life study with RU and arranging FP based products for mass awareness
Component 2 (RU)				
11.09.20		Dr. Md. Harunur Rashid	Director, PIU-BARC, NATP-2	Visited research labs and product samples; Some advice given; action was taken accordingly.
19.12.2020	1	Dr. Md. Monirul Islam	Sub-Project Coordinator and MD, Fisheries, BARC, Farmgate, Dhaka	Established effective coordination between the components
12.02.22	1	Dr. Md. Serajul Islam Munshi Mamunur Rahman	Consultant, (Social Safe Guard), Documentation Associate, PIU-NATP-2, BARC	Strengthen activities of sub project.

Date	No. of visit	Name/s of visitors	Addresses	Output
			Dhaka	
25.03.2022	1	1. Dipok Kumar 2. Md. Hasan Mahmud	Monitoring associate& Capacity dev. Associate, PIU, NATP. BARC	Deviation of activities identified and mitigated
18.05.22	1	Dr. Saleh Uddin Ahammed	Consultant PBRG sub projects (Fisheries) BARC	Suggestion for technical improvement of data analysis followed and improved

Plate 34. Desk and field monitoring of the sub-project activities

Plate 35. Field monitoring of sub-project activities at Depertment of Fisheries, RU

iii. Weather data, flood/salinity/drought level (if applicable) and natural calamities

Three years average information on weather for Noakhali region (2019 -2022)

Parameters	Seasons						Remarks
	Pre-Monson (January – April)		Monson (May – August)		Post Monson (Sept – December)		
	Max	Min	Max	Min	Max	Min	
Av. Rainfall (mm)	195	79	210	100	120	80	
Av. Temperature (^{O}C)	35	23	30	21	32	23	
Av. Humidity (%)	80	73	82	74	83	72	
Flood (year & category)	2013, moderate						
Av. Salinity (ppt)							
Draught	Occasionally						
Natural calamity (Frequency & category)	3, moderate						

Regional metrological office, Chattogram Division.

Three years average information on weather for Lakshmipur region (2019 -2022)

Parameters	Seasons						Remarks
	Pre-Monson (January – April)		Monson (May – August)		Post Monson (Sept – December)		
	Max	Min	Max	Min	Max	Min	
Av. Rainfall (mm)	190	75	180	110	130	70	
Av. Temperature (^{O}C)	34	27	32	23	31	25	
Av. Humidity (%)	85	72	80	73	81	75	
Flood (year & category)	2020, moderate						
Av. Salinity (ppt)							
Draught	Occasionally						
Natural calamity (Frequency & category)	1, moderate						

Regional metrological office, Chattogram Division.

Three years average information on weather for Khagrachari region (2019 -2022)

Parameters	Seasons						Remarks
	Pre-Monson (January – April)		Monson (May – August)		Post Monson (Sept – December)		
	Max	Min	Max	Min	Max	Min	
Av. Rainfall (mm)	200	78	330	120	200	80	
Av. Temperature (^{O}C)	35	29	31	24	30	27	
Av. Humidity (%)	90	79	81	74	85	78	
Flood (year & category)	2014, major						
Av. Salinity (ppt)							
Draught	Occasionally						
Natural calamity (Frequency & category)	2, minor						

Ref: Regional metrological office, Chattogram Division.

J. Sub-project Auditing (covers all types of audits performed)
Coordination component (BARC)

Types of audit	Major observation/ issues/ objections raised; if any	Amount of Audit (Tk.)	Status at the sub-project end	Remarks
Financial audit by FAPAD for the year 2019- 20	No objection raised, found all relevant documents updated as per guideline	701916.50	Financial management of the component found running smoothly till the end of the project No query or objection raised at any stage of operation by the audit teams	Financial management & project performance found satisfactory in all the audit cases
Financial audit by FAPAD for the year 2020- 21		478029.00		
Financial audit by FAPAD for the year 2021- 22		791609.00		

Component 1 (NSTU)

Types of audits	Major observation/ issues/objections raised; if any	Amount of Audit (Tk.)	Status at the sub-project end	Remarks
Financial audit by FAPAD for the year 2019-20	No major observation/objection or issues raised	661754.00	All minor observations observed negotiated and solved as per process No pending unsolved issue	Total financial management of the sub project component found satisfactory
Financial audit by FAPAD for the year 2020-21		7302325.00		
Financial audit by M.I. Chowdhury & C0. Year2020-21		7302325.00		
Financial audit by FAPAD for the year 2021-22		1795852.00		

Component 2(RU)

Types of audits	Major observation/ issues/objections raised; if any	Amount of Audit (Tk.)	Status at the sub-project end	Remarks
Financial by FAPAD for the year 2019-2020	No objection raised by the audit team	2123082.00	Financial management of the component found running smoothly till the end of the sub-project. No query or objection raised at any stage of the operation by audit teams.	Financial management and project performance was found satisfactory
Financial audit by FAPAD for the year 2020-2021		5856395.00		
Financial audit by M.I. Choudhury & Co. year2020-2021		5856395.00		
Financial audit by FAPAD for the year 2021-22		5423009.00		

K. Lessons learned

i) Top to bottom approach in the field resulted poor outcomes and less effective;
ii) Token money given to the homemakers played positive role for RCT product development;
iii) Gathered increased knowledge about the recipes of different value-added fishery products;
iv) Understand the impact of MAP packaging on increase of shelf-life of fish and fishery products stored at refrigerated temperature or at room temperature;
v) Commercial importance of MAP packagingof value-added fish products as means of better business and higher profitability;

L. Challenges

i) Working in the hilly part was challenging;
ii) COVID-19 situation drastically hinderedsub project activity. Procurement and subsequently research activities had been hampered invited a challenging situation for the research team to run activities as per plan;
iii) Delayed installation of tray sealer hampers the shelf-life study of few products due to time constraint which became challenging for the research team to complete all planned activities within a limited span of time;
iv) Successful operation of new machine with advance technology sometimes critical.
v) Collection of quality marine fishes to develop products at far away location like Rajshahi;
vi) Different barriers (*viz*,uncertain business interest, administrative, responsibility risks etc.) faced while marketing of the products in the superstores for consumer's acceptability study;

M. Suggestions for future planning

i) Developing a proper growth frindly supply chain establishment and marketing for FP.
ii) Technology can be disseminated to processors after validation.
iii) Entrepreneurship development involving small- and large-scale processors.

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Signature of the Coordinator	**Counter signature of the Head of the organization/authorized representative**
	
(Dr. Md. Rafiqul Islam)	***(Dr. Shaikh Mohammad Bokhtiar)***
Date: 20.12.2022	Date: 20.12.2022
Director (Nutrition)	***Executive Chairman***
Bangladesh Agricultural Research Council	***Bangladesh Agricultural Research Council***

Annexures

1. Coordination component (BARC)

Annexure – BARC: A

Recommendation of the inception workshop and status of action taken

Recommendations	Action Taken
Component 1 (NSTU)	
• Characterization and justification of selecting different geographical locations should be properly described;	Complied
• Considering the buying capacity of consumers, it is suggested to avoid high-cost fish species for product development study;	Followed as per suggestion
Component 2 (RU)	
• Marketing of RTE and RTC fish products by other different agencies like Bengal Meat etc. should verify and compare with the findings of present study;	This is not consistent with the specific objective of the sub project
• Special consideration should be given on quality and price of fish to be used for product development along with the buying capacity of the consumers;	Consideration given

Annexure – BARC: B

Recommendation of the half yearly workshops

Recommendations of the first halfyearly workshop	Action taken
General recommendation	
• Activity to ensure baseline survey and review of literature in time as per project plan	Completed as per plan
Recommendations of the second halfyearly workshop	
General recommendation	
• Finalize, approval and purchase of laboratory equipment and chemical reagents, recruitment of supporting staffs and their skill development training for smooth data collection, lab activities and data recording should be completed at the very initial stage of all field and lab activities	Attempt taken by the sub project components in time.
Recommendations of the third halfyearly workshop	
Component 1 (NSTU)	
• The total presentation was informative. But in all most all cases the format was deviated and it was not developed as per PCR format;	
• Research highlights should be elaborative and objective wise.	
• Research methodology and result discussions should be placed under different chapter heads instead of present style preparation.	

• Table on training should be provided with required information as per format;	Followed all advices in draft PCR preparation
• Put information in detail in the audit reporting format	
• Information on desk and field monitoring not recorded in proper way. Name and designation of the monitoring team members in many places not mentioned properly.	
• Information on materials development and publications made under the sub project placed scattered in the presentation of the report. All should be under the content of same table with different sub-heads/sub-titles avoiding duplications;	
• Separate paras on lesson learned, challenges and suggestions for future planning not discussed in the presentation at all;	
Component 2 (RU)	
• Status of the technology as shown in the technology generation table - no need to include;	Followed all advices in draft PCR preparation
• Output of desk and field monitoring information should be descriptive;	
• Full information should be provided in the audit reporting table;	
• Product popularize and marketing problem due to lack of bi-lateral agreements/contract between the producers and entrepreneurs may be considered as an challenge towards the sustainability of the efforts of product development and marketing;	------
• Product marketing trials should be ended by the mid of the June 2022;	Completed as per plan

Annexure – BARC: C

Recommendation of the annual workshop

Recommendations of the first annual workshop	Action taken
Component 1 (NSTU)	
• Source and name of fish species used for fish powder preparation are to be added in the report;	Full list added
• Nutritional composition of fish powder and additional ingredients (if any) to enrich the nutritive quality of fish-based products are to be focused clearly;	Discussed and focused clearly in the draft report
Component 3 (RU)	
• More nutrition potential fish species in addition to Tilapia recommended for trial to develop value added fish products;	Other fish species like Pangas, tune, sardine mackerel also added under the experiment for various different fish product development
• The preparation process of fish ball/crackers should be more scientific. Proximate composition of each item of product particularly protein content and very high level of	Followed advices

ash should be maintaining at standards applicable for human consumption;	
• Handmade products shown in the presentation looked products irregular shape. Suggested to use available simple mechanical device for better regular shape of the products;	Will be followed in commercial production process
Recommendations of the second annual workshop	**Action taken**
General comments	
• For introduction of products and establishment of marketing system (by both NSTU and RU components), development of entrepreneurship should be encouraged through establish linkage between the agents (producer and sellers)	Initiative taken and partial achievement done
• As per revised activity plan of the NSTU component, pre-clinical trial to evaluate the effect of nutrient rich Chapila fish supplement in anemic mice model experiment by March'22 at the latest.	Completed intime
• Suggested to work NSTU & RU jointly for shelf life study of fish powder. RU will extend necessary cooperation to the NSTU component in this regard.	Necessary cooperation extended to NSTU by the RU component
• Reflection of BCR values for each individual product in case of RU component suggested;	Followed
• Perfectness of volume of salt application in Mackerel Sausages should be re-examined;	Reexamined and adjusted
• Suggestion made for consideration of alternate ingredients for reducing the content level (present 60% to less) of carbohydrate in Tuna Crackers by RU component;	Followed to maintain the standard carbohydrate level in the product

Annexure – BARC:D

Recommendation of the coordination meetings

Two virtual central coordinationmeetings at BARC	
Component 1 (NSTU)	
• Covering so far more than nine months of its implementation, the progress of the component project is still at its very initial stage. Addition effort is required to complete the project activities as per stipulated time frame**;**	Necessary initiative taken and significant progress achieved within the reporting period
• Base line of the project to understand the intra-household food consumption, food allocation and nutritional outcomes of adolescent girls is still to complete. This survey and analysis works should be done within a shortest possible time (possibly by next 45 days) maintaining all sorts of safety measures for disease contamination prevention;	
• To address the specific objectives 2 & 3, development of ready to use fish based nutrition - dense products and screening of the best suited fish-based products to minimize the nutritional gaps should be started immediately providing special effort to complete the whole work within the last month of this year (2020);	Followed and done

Component 2 (RU)	
• This component project is also at its very initial stage of progress compare to time covered. Addition effort is required to complete the project activities as per stipulated time frame**;**	Initiative taken as per suggestion. Completed almost all te suggested activities but the procurement of shelf life study instrument due to technical problem. However, it is now already under process
• Procurement process for purchasing of vital equipment for RTC and RTE product development and packaging should be completed within next three months, positively. This was supposed to complete by March'20;	
• Development ofRTC products like, fish ball, battered & breaded fish, fish marinades, fish sausage etc. and RTE product like fish crackers and determination of overall shelf-life of the products under different storage temperature and finally to know the consumer's acceptability of MAP packaged fishery products is the vital part of this component project. Upon completion of the procurement of equipment, thrust should be given for immediate starting of all sorts or laboratory work, completion of that should not exceed December'2020;	
• Meanwhile, the PI is suggested to complete the base line study of the project component that should be incorporated in the coming annual report	
Recommendation of the 3rd coordination meeting	
Component 1 (NSTU) The following remaining activities of the sub-project component should be completed within the possible shortest time. Activities are:	All recommended activities completed by June 2022 as per revised time plan of the sub project
• Assessment of sensory, chemical and nutritional profile of Chapila fish powder;	
• Completion of preclinical trial to evaluate the effect of nutrient-rich Chapila fish supplement in anemic mice model;	
• Evaluation of consumer perceptions level on the PBRG ready to use Fish power consumption.	
• Attempt may be taken to study the shelf life of Fish powder with the available facility of the sub project	
Component 2 (RU) The following remaining activities of the sub-project component should be completed within the possible shortest time as mentioned against each activity. Activities are:	All recommended activities completed by June 2022 as per revised time plan of the sub project
• Completion of all procurement activities by January 2022;	
• Shelf-life study, other than previously studied 9 products, production of new other products (2+5 products) and their shelf-life study should also be completed by January 2022;	
• Product marketing and consumers preference should be completed by March, 2022;	

Recommendation of the 4th coordination meeting	
• Decision was taken to organize the two days workshop by the Coordination component within the first half of Oct'22; • The sub-project will be presented by Prof. Dr. Kazi Ahsan Habib of SAU, Principal Investigator on behalf of the other sub-project components; • Coordination component will prepare a list of expert members, general participants, session Chairman, Chief guest/Special guest along with a draft copy of workshop program; • The two days workshop will be held in the auditorium of the Training building of BARC. Coordination component will ensure the proposed venue;	PCR workshop held smoothly on 24-25 Oct'22 under the organizer ship of the Coordination component following all the instruction of the respective meeting.
Draft PCR review workshop comments	
General comments	
• Updating of financial, procurement and audit reports of all the sub-project components including the Co-ordination component	Complied
• Lesson learned and challenges of the report are not presented properly. In few places reforming of sentences are requires;	Revision of the section done
Component 1 (NSTU)	
• Biomarkers study information in the present report is incomplete. It requires rewriting of the total section including all parameters.	Necessary revision of the respective section done
Component 2(RU)	
• Benefit -cost information of various fishery products developed under the study is not shown in the report. It is suggested to include this.	Included in the final draft of PCR

Annexure RU:1

Office of the Principal Investigator

"Development of Fish-based Food Products and Extension of Shelf Life to Enhance Nutritional Security'

PIU-BARC, NATP-2, PBRG, Sub-Project No. 157

Department of Fisheries

University of Rajshahi, Rajshahi-6205

Questionnaire for Retailers (Superstore manager) about fishery products available in the Superstores

১। সুপারস্টোরের নাম ও স্থানঃ

২। তথ্য প্রদানকারী ব্যক্তির নাম ও ফোন নম্বরঃ

৩। ক্রেতারা কি ধরণের মাছ/মাংস পছন্দ করে? (পছন্দক্রম)

ক) বরফজাত (Iced); খ) শীতলীকৃত তাপমাত্রায় (Refrigerated); গ) হিমায়িত (Frozen)

৪। এখানে রান্নার উপযুক্ত (Ready-to-Cook) মাছের কি কি মূল্য-সংযোজিত দ্রব্য (value-added products) পাওয়া যায়? (ব্র্যান্ডসহ মূল্য (গ্রাম/টাকা) তালিকা)

বল, স্টিক, ফিঙ্গার, নাগেটস্, সসেজ, বার্গার, সামুচা, সিঙ্গারা , অন্যান্য...

এর মধ্যে কোনগুলো সামুদ্রিক মাছের.....

৫। এখানে খাওয়ার উপযুক্ত (Ready-to-Eat) কি কি মৎস্যজাত মূল্য-সংযোজিত দ্রব্য পাওয়া যায়?

৬। মাংসজাত কি কি মূল্য-সংযোজিত দ্রব্য পাওয়া যায়? (ব্র্যান্ডসহ মূল্য (গ্রাম/টাকা) তালিকা)

বল, স্টিক, ফিঙ্গার, নাগেটস্, সসেজ, বার্গার, সামুচা, সিঙ্গারা, অন্যান্য...

৭। আমদানিকৃত মাছ/মাংসের কি কি মূল্য-সংযোজিত দ্রব্য পাওয়া যায়? (ব্র্যান্ডসহ মূল্য (গ্রাম/টাকা) তালিকা)

৮। ক্রেতার চাহিদা অনুসারে মাছ/মাংসের মূল্য-সংযোজিত দ্রব্যের পছন্দক্রম কি হতে পারে?

১) ২) ৩) ৪) ৫) ৬)

৯। কত ওজনের মূল্য-সংযোজিত দ্রব্য বেশি বিক্রি হয়?

ক) ১০০ গ্রা; খ) ২৫০ গ্রা; গ) ৫০০ গ্রা; ঘ) ১ কেজি; ঙ) অন্যান্য....

১০। শীতলীকৃত / বরফজাত কোন মূল্য-সংযোজিত দ্রব্য আছে কি না?

যদি হ্যাঁ হয়, তাহলে কি কি?

যদি না হয়, তাহলে না থাকার কারণ কি কি হতে পারে?

ক) স্বল্প স্থায়িত্বকাল; খ) ক্রেতার চাহিদা কম; গ) সরবরাহকারি কোন প্রতিষ্ঠান নাই; ঘ) অন্যান্য ...

১১। শীতলীকৃত / বরফজাত মূল্য-সংযোজিত দ্রব্য (স্থায়িত্বকাল ২-৪ সপ্তাহ) যদি সরবরাহ করা হয়, তাহলে ক্রেতারা কি ক্রয় করবেন? হ্যাঁ / না

যদি না হয় , তাহলে ক্রয় না করার কারণ কি কি হতে পারে?

ক) স্থায়িত্বকাল কম; খ) প্রচলিত নয়; গ) গুণগত মান খারাপ; ঘ) অন্যান্য ...

১২। মূল্য-সংযোজিত দ্রব্যের ক্ষেত্রে নিচের কোন ধরণের দ্রব্য বেশি বিক্রি হবে বলে মনে হয়?

ক) বরফজাত (Iced); খ) শীতলীকৃত তাপমাত্রায় (Refrigerated); গ) হিমায়িত (Frozen)

১৩। সুপারস্টোরে শীতলীকৃত / বরফজাত মূল্য-সংযোজিত দ্রব্যের (স্থায়িত্বকাল ২-৪ সপ্তাহ) বাজারজাতকরণের সম্ভাবনা আছে কি না? হ্যাঁ / না

যদি না হয়, তাহলে তার কারণ কি কি হতে পারে?

১৪। শীতলীকৃত / বরফজাত মূল্য-সংযোজিত দ্রব্যের প্রচলনের ক্ষেত্রে আপনার কি পরামর্শ?

তথ্য সংগ্রহকারীঃ

মোবাইল: + ৮৮-০১৭১২৬৯৭২৫১
ফ্যাক্স: + ৮৮-০৭২১-৭৫০০৬৪
ই-মেইল: tariqrubd@gmail.com

Cell-Phone: +88-01712697251
Fax: +88-0721-750064
E-mail: tariqrubd@gmail.com

Annexure RU: 2

Office of the Principal Investigator

"Development of Fish-based Food Products and Extension of Shelf Life to Enhance Nutritional Security"

PIU-BARC, NATP-2, PBRG, Sub-Project No. 157

Department of Fisheries

University of Rajshahi, Rajshahi-6205

মডিফাইড এটমোস্ফিয়ার প্যাকেজিংকৃত (MAP) মূল্য-সংযোজিত মৎস্যজাত দ্রব্যের ভোক্তা পর্যায়ে গ্রহণযোগ্যতা নিরুপন

ক) আপনার ক্রয়কৃত দ্রব্যের নাম:

খ) পারিবারিক তথ্যাবলীঃ

১. উত্তরদাতার নাম:..... ২. বসবাসের এলাকার নাম: .....

৩. আপানার সহ পরিবারের অন্যান্য সদস্যদের তথ্য-

ক্রম	নাম (দিতে ইচ্ছুক না হলে x, y, z...লিখুন)	সম্পর্ক	লিঙ্গ (নারী/পুরুষ)	বয়স	শিক্ষাগত যোগ্যতা	পেশা	মাসিক আয়
১							
২							
৩							
৪							
৫							

গ) দ্রব্যের তথ্যাবলীঃ

১. আপনি কি ধরনের মাছ কিনতে পছন্দ করেন? ক) বরফজাত (Iced)/শীতলীকৃত (Chilled) খ) হিমায়িত (Frozen)

২. আপনি কি বাজার থেকে কোন মূল্য-সংযোজিত মাংসজাত দ্রব্য (যেমন- বল, নাগেটস, সসেজ) ক্রয় করেন? ক) হ্যাঁ খ) না, যদি হ্যাঁ হয়, দ্রব্যের নাম উল্লেখ করুন.....

৩. আপনি কি বাজার থেকে কোন মূল্য-সংযোজিত মৎস্যজাত দ্রব্য (যেমন- বল, ফিঙ্গার, সসেজ) ক্রয় করেন? ক) হ্যাঁ খ) না
যদি হ্যাঁ হয়, দ্রব্যের নাম উল্লেখ করুন.....

৪. আপনি কি ধরনের মূল্য-সংযোজিত দ্রব্য কিনতে পছন্দ করবেন?
ক) শীতলীকৃত (Chilled)/ রেফ্রিজারেটেড (Refrigerated) খ) হিমায়িত (Frozen)

৫. আপনি কি বাজারে কখনো কোন শীতলীকৃত (Chilled)/ রেফ্রিজারেটেড (Refrigerated) মূল্য-সংযোজিত দ্রব্য খুঁজে পেয়েছেন?
ক) হ্যাঁ খ) না

৬. আপনি কোন ধরনের মাছের মূল্য-সংযোজিত দ্রব্য কিনতে পছন্দ করবেন? ক) স্বাদুপানির মাছ খ) সামুদ্রিক মাছ

৭. আপনি কি মডিফাইড এটমোস্ফিয়ার প্যাকেজিং (MAP) সম্পর্কে শুনেছেন? ক) হ্যাঁ খ) না

৮. ক্রয়কৃত দ্রব্যের প্যাকেজিং কি আপনার পছন্দ হয়েছে? ক) হ্যাঁ খ) না
যদি না হয়, অনুগ্রহ করে কারনটি উল্লেখ করুন.....

৯. কোন সাইজের প্যাক কিনতে পছন্দ করবেন? ক) ১০০ গ্রাম খ) ১৫০ গ্রাম গ) ২০০ গ্রাম ঘ) ২৫০ গ্রাম
ঙ) ৫০০ গ্রাম চ) .....গ্রাম?

১০. আপনি কি মনে করেন, এই ধরনের মূল্য-সংযোজিত দ্রব্য বাজারে নিয়ে আসা দরকার? ক) হ্যাঁ খ) না
যদি না হয়, অনুগ্রহ করে কারনটি উল্লেখ করুন.....

১১. এই মূল্য-সংযোজিত মৎস্যজাত দ্রব্য সম্পর্কে আপনার কি কোন পরামর্শ আছে? .....

১২. অনুগ্রহ করে নিম্নের ফর্মটি পূরণ করুন: মাছের পণ্যের প্যানেল পরীক্ষা (৯ পয়েন্ট হেডোনিক স্কেল)

ক্রম:	বৈশিষ্ট্য	অত্যন্ত পছন্দ	খুব বেশি পছন্দ	মাঝারি পছন্দ	সামান্য পছন্দ	নিরপেক্ষ	সামান্য অপছন্দ	মাঝারি অপছন্দ	খুব বেশি অপছন্দ	অত্যন্ত অপছন্দ
		৯	৮	৭	৬	৫	৪	৩	২	১
১	রঙ (Color)									
২	গন্ধ (Flavor)									
৩	স্বাদ (Taste)									
৪	গঠন (Texture)									
৫	সামগ্রিক চেহারা (Overall appearance)									

অনুগ্রহ করে এই ফর্মটি পূরণ করে হার্ড কপি বা স্ক্যান কপি বা ফটো হিসাবে নিচের ঠিকানায় পাঠিয়ে দিনঃ

Dr. Md. Tariqul Islam
Principal Investigator & Professor, Department of Fisheries, University of Rajshahi
Mobile Phone/WhatsApp: +88-01712697251. Email: tariqrubd@gmail.com

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