Marine lizardfish (*Harpadon nehereus*) meal concentrate in preparation of ready-to-eat protein and calcium rich extruded snacks

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Abstract Bombay duck (*Harpadon nehereus*) a high protein fish, is one of the significant estuarine fish of the West Coast of India. Apart from being rich in protein the fish is also a store house of important minerals. This study was conducted to determine the physicochemical properties of the fish meal and utilize it to produce protein rich extruded snacks by twin screw extrusion technology at optimized parameters of 110 °C temperature, 16% moisture and 350 RPM screw speed. Protein of the fish meal concentrate was found to be around 54 g/100 g, mineral content particularly calcium around 2100.32 mg/100 g, phosphorous 240 mg/100 g and substantial amount of potassium, sodium, iron and zinc. Amino acid profile revealed presence of some essential amino acids. 1-D gel electrophoresis showed presence of low and medium kDa proteins ranging between 10 and 50 kDa. Functional and physicochemical property studies of extrudates revealed that 15% fish meal incorporation gave highest expansion ratio of 3.46. Addition of Bombay duck fish meal to extruded snack preparations had resulted in good taste and texture. The protein contents of the products ranged from 9.35 to 21.00 g/100 g. The results indicated that fish meal can be useful for preparing low cost protein rich food.

Keywords Bombay duck · Fish meal concentrate · Amino acid profile · SDS-PAGE · Texture profile analysis · Extruded snacks

Introduction

Fish is an exceptional source of major nutrition due to its high protein content and an incomparable source of lipid containing omega-3 fatty acids, especially, eicosapentaenoic acid and docosahexaenoic acid. At the same time, fish is also a good source of both fat soluble and water soluble vitamins and minerals like calcium, phosphorous, iron, zinc, iodine, selenium, potassium and sodium. Among the various protein sources available, fish protein is invariably one of the most significant sources of protein. Many fish proteins have been studied and used in food product development.

Harpadon nehereus or Bombay duck is a marine lizardfish belonging to phylum Chordate and distributed widely in the Indo-Pacific Ocean, Bay of Bengal and South China Sea. In India, this fish is largely available between September to June, and the annual catch is about 100,000 metric tonnes (Kakatkar et al. 2003). The fish is known for its high nutritional value and delicious taste, with up to 70% of protein and 1500–2500 mg/100 g calcium, on dry weight basis. The moisture content of the fish is, however, very high and sometimes ranges up to 90% making it highly perishable, this also makes the fish muscle proteins labile in nature. The fish is usually subjected to traditional drying method by exposing to sun to produce dried fish, fishmeal and other products (Jin et al. 2012; Kakatkar et al. 2003). Most of the reported work are based on drying techniques and nutritional properties of dried fish; in another study Nazir and Magar (1965) studied the chemical

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composition of Bombay ducks (*Harpadon nehereus*) and changes occurring in the nutritive value of dried fish on storage. It is also reported that sometimes the excess fish are disposed off which causes ecological pollution (Jin et al. 2012); wasting of highly nutritious fish should not be continued, especially in a country where there is high rise of protein–energy–malnutrition (PEM). Therefore, utilization of wise and economical processing technique should be employed for increasing the shelf life and nutritional value of the fish. There has been a trend in increasing consumption of ready to eat products among people. Bombay duck, because of its characteristic flavor and large availability, can be quite suitable for preparation of value added protein rich food products. There is one reported work where Bombay duck was used in chutney preparation (Bhosale et al. 2014). However, chutney or pickle because of its high calorific value and high oil content is not readily accepted as a healthy food for consumers of all age group. Among the suggested strategies to lower the risk of worldwide rise in obesity and chronic non-communicable diseases, fish consumption is one of the major strategies. Production of healthy snacks by using fish or fish flour can increase the rate of fish consumption. A key element to increase fish consumption is to provide processed and manufactured fish products preserving the high nutritional value of fish and maintaining customer expectations. In this regard, it will meet both ends by combating PEM on one side and reducing prevalence of life-style related diseases like obesity and CVD on the other hand. In the last decade, many researchers have utilized fish or fish flour in preparation of fish crackers and fish based snacks. Extruded puffed corn–fish snack using minced meat of silver carp (*Hypophthalmichthys molitrix*) was carried out. This fish despite being one the most important cultured fish in the world is becoming unpopular due to abundance of tiny bones which makes it unfit for filleting and direct consumption (Shahmohammadi et al. 2014).

Considering the fact that extrusion technology is one of the most versatile and effective food processing technologies of the era present study deals with utilization of *Harpadon nehereus* meal with bone in preparation of protein and calcium rich rice–corn–fish puffed snack.

Materials and methods

Raw materials

Fresh Bombay duck (*Harpadon nehereus*) that are generally caught by trawl net along the coast of Bay of Bengal, India, were used for the study. Approximately 10 kg fish, immediately after harvest, were collected from local fish market, Howrah, India. The fishes were washed in fresh

water and iced in the ratio of 1:1 (fish:ice) and transported to the laboratory. Head and entrails were removed manually and washed with chilled water and stored in refrigerator ($< 0^{\circ}\text{C}$). Rice flour and yellow corn flour were purchased from B. M. Agarwal (Bhowanipur) Kolkata, India. Refined rice bran oil (RBO) was gifted by M/S Shethia Oil Mill (Shaktigarh, West Bengal, India). Seasoning material including spices (garlic powder, onion powder, ginger powder and green chilli powder) and salt were purchased from local grocery.

Preparation of fish meal concentrate (FMC)

Clean, beheaded, degutted fish with bones were cut into small pieces. Cut pieces were blanched in boiling water for 10 min under normal atmospheric pressure. Blanched fish was dried in a hot air oven (LASIN Itherm AI-7981) at $50\text{--}55^{\circ}\text{C}$. The dried fish mass was powdered in a domestic mixer (Jaipan, JP-899) having approximately 16,000 rpm rotating speed to get fish meal concentrate. The fish meal concentrate was packed in a jar after sieving and stored in a refrigerator at 4°C until further analysis and preparation of blend for extrusion.

Principal component analysis (PCA) and mineral estimation

Principal composition like moisture and total ash contents of fish meal concentrate were analyzed by standard procedures (AOAC 2006) and protein content was computed following method of Lowry et al. (1951). Fat was determined by method of Bligh and Dyer (1959). Carbohydrates were determined by difference, [Carbohydrate (%) = $100\% - (\text{moisture} + \text{ash} + \text{crude protein} + \text{crude fat})\%$]. Minerals like Iron, Phosphorous, Zinc, Calcium, Copper, Sodium and Potassium) were determined by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES, 6300 Duo) after HNO_3 wet digestion (Zodape et al. 2010). The extruded products were also subjected to principal component analysis, calcium and phosphorous estimation. The energy value for fish meal concentrate based snacks was calculated in kilocalories/100 g (Manzi et al. 2001).

Amino acid profile of fish meal concentrate

Amino acid composition was determined following method of Ishida et al. (1981) with minor modification. Fish meal concentrate was hydrolyzed with 6 N hydrochloric acid at 110°C under anaerobic condition for 24 h. The hydrolyzed samples were neutralized with 6 N NaOH and were derivatized using a kit (AccQ-Fluor Reagent, WAT052880, Waters). The derivatized sample was injected in high

performance liquid chromatography (HPLC) (1525, Waters) equipped with a c18 RP column and a fluorescence detector (2475, Waters). The amino acids were identified and quantified by comparing the retention times and peak areas of authentic amino acid standards (WAT088122, Waters). All data have been presented in triplicate as mean $\pm$ standard deviation.

SDS-PAGE 1-D profile

The molecular weight distribution of fish meal concentrate was observed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) according to the method of Laemmli (1970) with minor modification as reported by Park et al. (2016). The sample (10 and 15 μ g protein) was loaded on the precast gel (Bio-Rad Lab., USA) and subjected to electrophoresis at a constant current of 11 mA per gel using a Bio Rad POWER PAC 3000 cell. Coomassie brilliant blue was used to stain the Electrophoresed gel and de-staining was carried out using 25% methanol and 10% acetic acid until clear bands were visible. Molecular weight of protein bands was estimated using Precision Plus Protein Unstained Standards (10–250 kD, Bio-Rad Lab).

Preparation of FMC-flour blend

The blends were prepared using rice and yellow corn flour with fish meal incorporated at different levels. The conventional flours were chosen as source of cereal for co-extrusion with fish meal concentrate. These flours are reported to provide better plasticizing property and expansion to the product. The ingredients (cereal and fish meal concentrate) were equilibrated to room temperature and weighed according to the formulation as shown in Table 1, before mixing. All the ingredients were mixed in the mixer (NOVA NM-79WB) with the required quantity of water and salt, packed in a polythene bag and kept at room temperature for proper conditioning. This preconditioning procedure was employed to ensure uniform mixing and hydration and to minimize variability in the state of the feed material. The mixtures were sieved using a 40 μ m mesh screen before extrusion.

Extrusion cooking and optimization

Different feed mixes containing FMC (5–30%) were each equilibrated at different moisture (14, 16 and 18%) levels and extruded at different temperatures (100–120 °C) by feeding manually into hopper of Twin screw extruder (LAB Model EB-10, Basic Technology Pvt. Ltd, Kolkata, India). The barrel screws were revolved at 350 RPM for all the extruder runs. Rod-shaped extrudates through round-hole die (diameter of 3 mm) were collected and cut into

small pellet and kept into polythene bags and sealed separately. Table 1 shows the combination of variables chosen in terms of moisture and temperature for production of highly expanded extrudates with FMC addition. Optimum parameters for product development were selected on the basis of expansion ratio of the products. 16% feed moisture and 110 °C temp produced puffed snacks with higher expansion compared to others. From each sample obtained from the optimized extruded conditions, three random replications were taken for measurement of physicochemical analysis, functional properties, texture profile analysis, sensory acceptability and microbial studies. A control product of corn and rice flour blend without addition of fish meal was produced simultaneously under similar processing condition. The products were seasoned with 10% RBO (w/w) and 15% (w/w) spice mixture (green chilli powder, ginger powder, garlic powder, roasted cumin powder and salt) post extrusion.

Functional properties of extruded snacks

Moisture content was determined following the official method of Association of Official Analytical Chemists (AOAC 2006) and was expressed in terms of percentage. Physical properties like expansion ratio (ER) was carried out following method of Sawant et al. (2013); porosity (PO) was determined by calculating the particle density (PD) and bulk density (BD) (Deshpande and Poshadri 2011; Gujska and Khan 1990; Wang et al. 1999). Functional properties like water holding capacity (WHC), oil holding capacity (OHC), water absorption index (WAI), water solubility index (WSI), and bulk density (BD) were carried out following methods adapted by Deshpande and Poshadri (2011).

Instrumental color parameters

Powdered test samples were separately poured in a 50 $\times$ 12 mm glass petri dish and color was measured using Konica Minolta Color reader (CR-10). The color reader was calibrated with black and white color before use and reported as L*, a* and b* values. L* is a measure of lightness, a* represents the color range from green to red and b* represents the color range from blue to yellow. Color was measured in triplicate values per test sample.

Texture profile analysis

Texture profile of the extruded samples was carried out using a Texture Analyzer (Stable Micro Systems TA.XT Express Enhanced, UK) equipped with P/5 aluminium cylinder probe of 5 mm diameter. Randomly selected extruded sample was placed below the probe and

Table 1 Ingredient composition of formulated extruded blends (g) and optimization of parameters

Ingredients	CS	FMCS1	FMCS2	FMCS3	FMCS4	FMCS5	FMCS6
Ingredient composition of formulated extruded blends (g)							
Corn flour	70	66.5	63	59.5	56	52.5	49
Rice flour	30	28.5	27	25.5	24	22.5	21
FMC	0	5	10	15	20	25	30
Water	16	16	16	16	16	16	16
Set of FMC snack formulation			Moisture %		Temperature °C		
Optimization of parameters for preparation of extrudates using the above formulation							
1			14			100	
						110	
						120	
2			16			100	
						110	
						120	
3			18			100	
						110	
						120	

FMC fish meal concentrate, *FMCS1* fish meal concentrate based snack with 5% FMC, *FMCS2* fish meal concentrate based snack with 10% FMC, *FMCS3* fish meal concentrate based snack with 15% FMC, *FMCS4* fish meal concentrate based snack with 20% FMC, *FMCS5* fish meal concentrate based snack with 25% FMC, *FMCS6* fish meal concentrate based snack with 30% FMC, *CS* control snack

compressed twice and the texture profile in terms of hardness, springiness, fracturability gumminess and chewability value was recorded and analyzed by Texture Exponent LiteExpress software program (version 6.0). Measurements were carried out in triplicate for each sample.

Sensory studies of extruded snacks

All the experimental products along with control product were served to a panel of twenty members involving the post graduate students and research scholars, who knew the area of Food processing and nutrition science at the School of Community Science and Technology IIEST, Shibpur. They were asked to evaluate sensory attributes such as appearance, flavor, taste, texture and over all acceptability on a nine point Hedonic scale, wherein 9 stands for like extremely, 5 for neither like nor dislike and 1 for dislike extremely.

Microbial study

The microbial load was assayed in terms of total plate count, total fungus and mould growth, and pathogenic *E. coli* following the methods of APHA (2005). Microbial study was carried out for day 7, day 28, day 90 and day 120.

Statistical analysis

For the statistical analyses of data the Origin 8 Pro software for data analysis was used. Statistical analysis was performed using analysis of variance (ANOVA). All the analyses were carried out in triplicates and the mean values were reported. Comparison of the means across the group was done by Tukey test. Statistical analysis was repeated at least twice with the Origin pro 8 statistical software. The significant levels were determined at $p \leq 0.05$.

Results and discussion

Principal component analysis and mineral estimation

The raw fish has very soft and tender flesh with high water content; unlike other fish bones, its bones are soft too. The proximate values of dried fish meal concentrate are tabulated in Table 2. The moisture % in the dried fish meal concentrate was found to be 15.5%. Dried Bombay duck generally contains around 11–12% moisture as reported by Nazir and Magar (1965). Siddique et al. (2012) in another study found that dry Bombay duck contained around 24% moisture during winter and around 33% moisture during

Table 2 Principal component, mineral content and amino acid profile of Bombay duck fish meal concentrate (FMC)

Principal components (g/100 g)			Bombay duck FMC
Protein			54 ± 0.01
Carbohydrate			2.21 ± 0.02
Fiber			–
Ash			13.31 ± 0.31 (FMC)
			8.25 (dried flesh)
			15.00 (fish bone)
Fat			3.32 ± 0.47
Moisture (%)			15.5 ± 4.50
<i>Minerals</i>			
Calcium			2100.32 ± 0.08
Phosphorous			240 ± 0.30
Iron			18.47 ± 1.10
Potassium			542.99 ± 0.09
Magnesium			488.58 ± 0.44
Sodium			964.30 ± 0.06
Zinc			17.87 ± 0.09
Amino acid (g/100 g)	Bombay duck FMC g/100 g (db) (present study) ^a	Bombay duck g/100 g protein (Longvah et al. 2017)	Bombay duck g/100 g wet fish (Jin et al. 2012)
<i>Essential amino acids</i>			
Glutamic acid	8.25 ± 0.28	11.16	1.87
Glycine	4.90 ± 0.15	6.17	0.46
Histidine	1.20 ± 0.30	–	0.39
Arginine	5.08 ± 0.12	5.25	0.63
Threonine	6.26 ± 0.20	–	0.5
Proline	6.84 ± 0.54	6.14	0.44
Tyrosine	2.54 ± 0.24	4.23	0.28
Valine	0.21 ± 0.01	–	0.48
Methionine	0.21 ± 0.02	–	0.35
Lysine	2.32 ± 0.04	–	0.94
Isoleucine	0.20 ± 0.00	–	0.46
Leucine	2.70 ± 0.00	–	0.82
Phenylalanine	0.40 ± 0.01	–	0.41
TEAA=	41.07 ± 0.06	–	–
<i>Non-essential amino acids</i>			
Aspartic acid	10.07 ± 0.17	9.93	1.02
Serine	4.06 ± 0.36	4.5	0.4
Alanine	2.86 ± 0.11	5.95	0.68
TNEAA	16.97 ± 0.02	–	–
TAA	58.1 ± 0.00	–	–
TEAA/TNEAA	2.41 ± 0.01		

Results of principal components are mean ± SD. n = 3. All data are reported on dry basis (db)

^aResults of amino acids are mean ± SD, n = 3; TAA total amino acid, TEAA total essential amino acid, TNEAA total non-essential amino acid

monsoon. Protein content of fish meal concentrate (FMC) was found to be 54%. Bhattacharya et al. (2016) reported presence of around 53.91–56.33% protein in dried Bombay duck fish with different pre-treatments; similar findings were reported by Siddique et al. (2012). However, Nazir and Magar (1965) reported presence of around 84% protein in plain dried Bombay duck. High protein of the fish makes it a very good raw material for protein rich snack preparation. It is seen that around 3 g of fat is present in the dried FMC. Also very low amount of carbohydrate around 2.21 g/100 g was found in the fish meal concentrate, owing to fish being highly proteinaceous. Ash content of different fish parts was evaluated. Total ash content of the whole fish, fish flesh and fish bone was 13.31%, 8.25% and 15% respectively as tabulated in Table 2. Nazir and Magar (1965) accounted that dried Bombay duck contain about 9.6% total ash, and brine treated dried fish around 26–35% ash. Mineral content of the fish is remarkably high. Finding that the fish bones are remarkable source of minerals, the bones along with the flesh was used in FMC preparation and utilized for product formulation. Amount of calcium, iron, potassium, phosphorus were found to be in good amount in the FMC as shown in Table 2. Both the Calcium and Phosphorous content of the FMC are remarkable, Calcium content was found to be 2100.32 ± 0.08 mg/100 g and Phosphorous content was around 240 ± 0.3 mg/100 g.

Conventional flour like rice flour and yellow corn flour used for snack preparation were having carbohydrate content of 79.8 ± 0.26% and 77.6 ± 0.1%, Protein 7.24 ± 0.89% and 7.1 ± 0.09%, Fiber 2.4 ± 0.54% and 7.35 ± 0.23%, Ash 0.67 ± 0.22% and 1.42 ± 0.77%, Fat 1.42 ± 0.9% and 3.92 ± 0.09%, Moisture 10.8 ± 0.1% and 10.45 ± 0.9%, respectively.

Amino acid profile

Amino acid profile of the fish meal concentrate showed presence of essential amino acids like Glutamic acid, Glycine, Arginine, Proline, Tyrosine, Methionine and Leucine, which are known to be essential in human nutrition (Table 2). The various essential amino acids that are present in the FMC are functional in nature. Arginine was found to be around 5.08 g/100 g FMC, Lysine was found to be 2.32 g/100 g FMC. Among the functional essential amino acids, Glutamic acid was present in highest amount followed by Proline > Arginine > Glycine > Leucine > Tyrosine > Methionine. Longvah et al. (2017) reported presence of comparatively higher amount of Glutamic acid and Glycine. Among the aromatic amino acids Tyrosine was found to be 2.54 g/100 g, however, Tryptophan was not determined in the present study. Among the non-essential amino acids Aspartic acid content was found to be

the highest (10.07 g/100 g FMC) followed by Serine and Alanine. Total Essential Amino Acid (TEAA) was found to be 41.07 ± 0.06 g/100 g and Total Non-essential Amino Acid (TNEAA) was found to be 16.97 ± 0.02 g/100 g. TEAA/TNEAA ratio was found to be 2.41.

SDS-PAGE 1-D profile

SDS-PAGE patterns representing the distribution of protein on the basis of molecular weight of *Harpadon nehereus* FMC were shown in Fig. 1. The first lane (M) showed the distribution of standard marker protein, having protein moieties of 10–250 kDa. *Harpadon nehereus* meal concentrate gave discrete protein bands with a molecular weight (MW) ranging from 10 to 75 kDa; band obtained of 50 kDa was dominant in FMC. Band of 37 kDa was observed indicating presence of tropomyosin, similar bands were observed by Saha (2014) in Hoki acid treated fish. Other protein bands in range of 10–20 kDa, were also observed. Three light chain myosin bands are clearly visible in the electrophoretic representation between 10 and 20 K. Similar bands were observed and reported by Carache et al. (1995). Bands of Myosin light chains are found in range between 15 and 30 kDa as reported by Sotelo et al. (2000). The fish meal under the present study did not show any protein bands of higher molecular range (100–250 kDa) as can be seen from the electrophoretic representation, indicating absence of myosin heavy chains.

Functional properties

In extrusion cooking, expansion is the primary quality parameter associated with product crispiness, water absorption, water solubility and crunchiness. Protein affects expansion through their ability to effect water

Fig. 1 SDS-PAGE 1-D profile of *Harpadon nehereus* Fish Meal Concentrate (FMC). M Marker (standard protein marker), FMC fish meal concentrate

distribution in the matrix and through their macromolecular structure and confirmation. From the result of expansion ratio (ER) of the products a general trend of lowering of ER was noted with increased level of fish meal. However, from the statistical analysis (Table 3), it was revealed that the Fish meal concentrate snack-3 (FMCS3) with around 15% fish meal incorporation showed a better ER of (4.36 ± 0.20) than other samples and even higher than the control extruded snacks, indicating better optimization of ingredient composition. On the other hand FMCS6 has less ER which contained more amount of fish flour. Similar findings were reported (Deshpande and Poshadri 2011; Sawant et al. 2013).

Oil absorption capacity (OAC) was found to be more for FMCS2 approximately 398%. However, higher absorption of oil may be attributed to presence of less fat and more amount of crude fiber.

The water holding capacity (WHC) was highest for FMCS2 (approximately 701%) compared to other samples. This could be due to higher level of cereal starch and crude fiber in the composite flour of that particular blend. Similar results were observed (Deshpande and Poshadri 2011; Shirani and Ganesharanee 2009).

The tap density decreased with optimum level of protein in the composite flour. The Tap density was more for product FMCS4 (approximately 0.52 g/c^3) compared to other samples. Similar results were observed by Deshpande and Poshadri (2011). The true density (TD) was maximum for the FMCS2 approximately 1.62 g/c^3 than other samples. The TD increased with cereals starch in extrudates. Related findings were quoted by Ding et al. (2005). Bulk density (BD) is a very important parameter in the production of expanded and formed food products. Bulk density, which considers expansion in all directions, is an index of the extent of puffing. BD is expressed in g/cm^3 . More crude fiber in the composite flour sample also results in higher BD. BD was found to be least for both FMCS5 and FMCS6 ($0.08 \pm 0.00 \text{ g/c}^3$) as compared to other samples, while maximum BD was observed for FMCS2 $0.52 \pm 0.3 \text{ g/c}^3$. Parallel type of results were observed by Deshpande and Poshadri (2011) and Sawant et al. (2013).

Porosity (PO) measures the air pockets or gas cells with variable pore size and number that are created during extrusion giving expanded products with lower BD. Yanniots et al. (2007) suggested that PO created during extrusion can be used to describe the expansion properties of the extruded products. The maximum and minimum PO of extrudates obtained in this study was 49.55 ± 0.00 for product FMCS2 and 2.72 ± 0.57 for product FMCS6 as can be seen from Table 3. Low PO of product FMCS6 might be due to high amount of protein from fish meal which prevented expansion resulting in formation of non-porous textured product.

Table 3 Functional properties, principal component and mineral content of FMC incorporated snacks

Parameters	Samples						
Functional properties	CS	FMCS1	FMCS2	FMCS3	FMCS4	FMCS5	FMCS6
ER	3.71 ± 0.00	3.47 ± 0.04 ^b	3.38 ± 0.05 ^c	4.36 ± 0.20 ^a	3.87 ± 0.05 ^c	2.93 ± 0.03 ^c	2.27 ± 0.00 ^d
OAC (%)	298.40 ± 0.40	367.8 ± 0.80 ^c	398.11 ± 0.1 ^c	286.25 ± 0.25 ^a	210.9 ± 0.10 ^e	240.90 ± 0.10 ^d	275.26 ± 0.25 ^b
WHC (%)	640.50 ± 0.50	631.90 ± 0.90 ^a	701.50 ± 0.5 ^b	510.50 ± 0.50 ^c	428.9 ± 0.10 ^d	400.40 ± 0.4 ^d	404.40 ± 0.40 ^d
Tap density (g/c ³)	0.36 ± 0.00	0.26 ± 0.00 ^b	0.28 ± 0.00	0.49 ± 0.00 ^a	0.52 ± 0.00 ^a	0.51 ± 0.00 ^a	0.51 ± 0.00 ^c
TD (g/c ³)	1.04 ± 0.01	0.87 ± 0.00 ^d	1.62 ± 0.02 ^d	0.93 ± 0.05 ^a	1.25 ± 0.04 ^c	1.25 ± 0.00 ^d	0.99 ± 0.00 ^b
PD (g/c ³)	0.16 ± 0.00	0.30 ± 0.00 ^c	0.65 ± 0.00 ^d	0.20 ± 0.00 ^a	0.20 ± 0.00 ^a	0.09 ± 0.00 ^b	0.08 ± 0.00 ^b
BD (g/c ³)	0.12 ± 0.00	0.21 ± 0.00 ^c	0.52 ± 0.34 ^a	0.15 ± 0.00 ^b	0.16 ± 0.00 ^b	0.08 ± 0.00 ^b	0.08 ± 0.00 ^c
PO	22.01 ± 0.01	31.26 ± 0.02 ^c	49.55 ± 0.00 ^c	21.54 ± 0.14 ^a	18.41 ± 0.00 ^c	10.68 ± 0.01 ^d	2.72 ± 0.57 ^b
WAI %	6.51 ± 0.01	6.54 ± 0.03	6.71 ± 0.01 ^b	6.64 ± 0.02 ^a	3.67 ± 0.01 ^d	3.64 ± 0.03 ^c	3.51 ± 0.00 ^c
WSI %	0.10 ± 0.00	0.10 ± 0.00 ^a	0.09 ± 0.00 ^b	0.09 ± 0.00 ^c	0.12 ± 0.00 ^c	0.14 ± 0.00 ^d	0.13 ± 0.00 ^d
MFR g/s	4.80 ± 0.00	4.80 ± 0.00	4.80 ± 0.00	4.77 ± 0.00 ^a	4.77 ± 0.00 ^b	4.68 ± 0.01 ^c	4.67 ± 0.00 ^c
<i>Color values</i>							
L*	67.40 ± 0.10	65.60 ± 0.30 ^b	62.85 ± 0.05 ^a	61.8 ± 0.10 ^d	60.12 ± 0.02 ^d	58.80 ± 0.00 ^d	57.72 ± 0.28 ^c
a*	9.22 ± 0.08	4.25 ± 0.15 ^b	6.08 ± 0.07 ^a	2.95 ± 0.05 ^c	3.17 ± 0.07 ^c	3.25 ± 0.05 ^c	2.10 ± 0.10 ^c
b*	33.45 ± 0.15	42.27 ± 0.37 ^a	36.45 ± 0.05 ^a	23.45 ± 0.05 ^c	39.70 ± 0.10 ^b	40.15 ± 0.15 ^b	40.15 ± 0.02 ^b
<i>Principal component (g/100 g)</i>							
Moisture	8.35 ± 0.01	8.84 ± 0.01 ^a	9.04 ± 0.03 ^a	9.29 ± 0.02 ^b	10.03 ± 0.02 ^c	10.45 ± 0.04 ^b	11.05 ± 0.01 ^d
Ash	1.38 ± 0.02	2.09 ± 0.01 ^a	3.27 ± 0.01 ^b	3.53 ± 0.01 ^b	3.94 ± 0.02 ^b	4.44 ± 0.02 ^c	5.14 ± 0.10 ^c
Protein	7.20 ± 0.01	9.34 ± 0.01 ^a	11.27 ± 0.02 ^a	14.07 ± 0.03 ^b	16.53 ± 0.01 ^c	18.82 ± 0.01 ^d	21.01 ± 0.02 ^c
Fat	2.36 ± 0.01	2.86 ± 0.01 ^a	3.09 ± 0.01 ^b	3.13 ± 0.01 ^b	3.25 ± 0.05 ^a	3.56 ± 0.02 ^b	3.80 ± 0.00 ^c
Fiber	5.81 ± 0.01	5.34 ± 0.02 ^a	4.95 ± 0.01 ^b	4.61 ± 0.00 ^c	4.10 ± 0.03 ^c	3.91 ± 0.00 ^d	3.41 ± 0.01 ^d
Carbohydrate	75.02 ± 0.02	72.84 ± 0.02 ^a	69.59 ± 0.02 ^b	66.20 ± 0.01 ^c	62.39 ± 0.01 ^c	58.42 ± 0.01 ^d	55.13 ± 0.02 ^d
Energy (Kcal)	350.20 ± 0.23	354.56 ± 14 ^b	351.32 ± 0.31 ^a	349.26 ± 0.05 ^a	344.93 ± 0.50 ^b	341.07 ± 0.16 ^c	338.88 ± 0.22 ^c
<i>Minerals (mg/100 g)</i>							
Calcium	9.61 ± 0.03	102.00 ± 0.50 ^a	212.32 ± 0.04 ^b	318.02 ± 0.10 ^b	417.78 ± 0.13 ^d	522.21 ± 0.21 ^c	624.32 ± 0.06 ^a
Phosphorous	18.08 ± 0.04	30.27 ± 0.21 ^b	41.11 ± 0.11 ^a	53.00 ± 0.05 ^a	65.87 ± 0.15 ^b	73.98 ± 0.20 ^c	87.55 ± 0.50 ^c

Results are mean ± SD, n = 3, Mean Values having different superscript letter in rows are significantly different ($p \leq 0.05$)

FMCS1 Fish meal concentrate based snack with 5% FMC, *FMCS2* Fish meal concentrate based snack with 10% FMC, *FMCS3* Fish meal concentrate based snack with 15% FMC, *FMCS4* Fish meal concentrate based snack with 20% FMC, *FMCS5* fish meal concentrate based snack with 25% FMC, *FMCS6* fish meal concentrate based snack with 30% FMC, *CS* control snack

Water Absorption Index is the volume occupied by the starch of the extruded products after they swell in presence of surplus water, this maintains the integrity of starch in water medium. Water Solubility Index denotes the rate and degree to which the element of flour material dissolves in water. WSI is used as a sign of degradation of molecular components, measures the extent to which starch gets converted during extrusion. It also measures the amount of soluble polysaccharide released from the starch after extrusion of flour (Ding et al. 2005). The WAI was found to be more for FMCS2 (6.71%) than other samples. Table 3 showed that the water absorption index of the extrudates increased with increase of rice and corn flour. The WSI was more for the FMCS5 (0.14%). WSI increases with increase in amount of fish flour. These results are more or less in

conformity with the observations made (Deshpande and Poshadri 2011; Sawant et al. 2013; Shirani and Ganesha-ranee 2009).

Mass flow rate (MFR) was minimum for FMCS6 (4.67 ± 0.00 g/s) followed by sample FMCS5 (4.68 ± 0.01 g/s), sample FMCS4 and FMCS3 (4.77 ± 0.00 g/s) and the rest 4.80 ± 0.00 g/s which is same as the control product. The variations in the MFR of extrudate samples were very less, due to constant maintenance of barrel temperature as well as moisture content in the feed mixtures. Yet the samples with high amount of fish meal showed slightly less MFR which might be due to slow rate of mass flow within the barrel due to high protein rich mixture. Proteins are known to have higher molecular weight and subsequently they make products

Fig. 2 **a** Texture profile analysis of FMC-based snacks and **b** sensory profile of FMC-based extruded snacks. *FMCS1* fish meal concentrate based snack with 5% FMC, *FMCS2*: fish meal concentrate based snack with 10% FMC, *FMCS3* Fish meal concentrate based snack with 15% FMC, *FMCS4* fish meal concentrate based snack with 20% FMC, *FMCS5* fish meal concentrate based snack with 25% FMC, *FMCS6* fish meal concentrate based snack with 30% FMC, *CS* control snack

(extrudates) heavy, bulky and at times hard as well which might result slowing down of the flow rate of the extrudates.

Color parameters

Color is a significant feature which is directly proportional to the acceptability of food products. It is an important physical property to report for extruded food products. Color parameter provide information about the degree of occurrence of browning reactions, extent of cooking and color degradation during the extrusion cooking (Ilo and Berghofer 1999). The lightness (L^*) (positive L^* is white and negative L^* is black) is an indication of the brightness. The lightness value of the products ranges from 57.72 to 67.40. Table 3 showed that the lightness of the control snack was highest and it decreased as the amount of FMC in snacks increased. Color parameter a^* , indicative of the redness of sample varied from 2.1 to 9.22. Dark color also develops during caramelization of sugar from the Maillard reaction. The yellowness (b^*) value of extruded products varied from 23.4 to 42.27. The change in yellowness during

extrusion cooking was most induced by the effects of non-enzymatic browning and pigment destruction reactions. All these differences could have been due to the shear forces generated during extrusion cooking which accelerated the chemical reactions between amino acids and reducing sugars (Maillard reaction) that take place during extrusion. Heat generated during extrusion cooking may affect the yellow color of corn (Guy 2001; Santosa and Sudaryono 2008; Sawant et al. 2013). The gradual increase from 0 to 30% of fish meal concentrate addition in extruded snacks caused a linear decrease in luminosity; 30% level showed L^* of 57.72 and 0% level showed L^* equal to 67.40. Similar observation was reported by Justen et al. (2011). This shows that the inclusion of fish meal concentrate (FMC) in corn–rice snacks diminished intensity of luminosity and yellowness.

Texture profile analysis

Texture is one of the most significant attributes that control the quality of a product, as in snacks obtained through thermoplastic extrusion. In extruded snacks, expansion is desired and puffed products are expected, and this is why texture plays an important role regarding the acceptability of snacks among consumers (Anton and Luciano 2007). Addition of FMC increased the hardness of the extruded snacks; as can be seen from Fig. 2a with every 5% increase in FMC the hardness showed a gradual increase. Extrudate hardness is strongly influenced by the protein content. Increased protein content in feed material produce more rigid network resulting in product hardness. Fracturability is the ability to break food into pieces when it is bitten using set of incisors as described by Paula and Conti-Silva (2014). In the present study products showed fracturability of the range 14.58–28.74 N. Fracturability is highly related with extrudate hardness and product with highest FMC incorporation showed highest fracturability. Springiness value of the product depicts that that the products are quite crunchy in nature. As described by Shah et al. (2012) springiness has inverse relation with crispiness of snacks, which means that product with lesser springiness value are crispier and hence preferred over other products having higher springiness. Higher springiness is observed in product with higher FMC content especially product FMCS5 and FMCS6 showed high springiness values compared to others. Gumminess of the products ranged between 0.35 and 1.12. According to Shah et al. (2012) chewiness is the behavior of snack food during biting or chewing. Maximum value for the chewiness (0.18) was noted in snack FMCS6 supplemented with 30 g of FMC followed by the snack FMCS5 supplemented with 25 g of FMC > FMCS4 and FMCS3 > FMCS2 > FMCS1.

Proximal analysis

In this study moisture content of the products was found to be the highest in experimental product FMCS6 and lowest in control product (Table 3). Product with high amount of FMC incorporation showed comparatively high moisture values. High moisture results in higher water activity (a_w). High moisture percentage in food products harbors more microbial growth. The moisture content of the experimental products ranged between 8.84 ± 0.00 to 11.05 ± 0.01 g/100 g. Mineral matter (ash), protein, fiber, carbohydrates and calorie were different for the snacks with different levels of FMC addition ($p < 0.05$). Ash is considered as the total mineral and or inorganic content of the sample. The ash % increased with increased FMC as can be seen from Table 3. The higher amount of ash obtained was 5.14 ± 0.10 (FMCS6). All the products showed good ash content ranging from 2.09 ± 0.01 to 5.14 ± 0.10 g/100 g. The higher ash of the product with increasing amount of FMC is owing to the fact that fish meal concentrate is rich in total mineral content.

The protein contents obtained were in the range from 11.27 ± 0.02 to 21.01 ± 0.02 g/100 g as tabulated in Table 3. Protein % increased with increased fish flour. The protein content of control snack was found to be 7.20 ± 0.01 g/100 g, whereas the addition of FMC increased the protein content. *Harpadon nehereus* fish meal concentrate contained approximately 54% protein. High protein content of 18.82 ± 0.01 and 21.01 ± 0.02 g/100 g of products FMCS5 and FMCS6, however, produced hard textured product, due to high protein content. In a study involving utilization of various fish flour in production of extruded snacks, Souza et al. (2015) reported that Tuna fish incorporated extruded snack had the highest protein percentage (11.85%), followed by the sardine and tilapia fish based snacks, while salmon fish incorporated snack had the lowest protein rates (9.21%), with each snack having 9% fish meal incorporation. This is almost similar with the present study, where around 10% FMC incorporation in extruded snacks resulted in 11.27% protein, as can be seen from Table 3, in FMCS2. In another study addition of 30% blanched dried Lizard fish (*Saurida tumbil*) powder resulted in extruded snack with highest protein content of around 28% (Ganesan et al. 2017). Proteins in fish muscles (in general animal protein) have higher biological value than plant protein with a balanced amino acid composition. Therefore, snacks enriched with FMC are an important source of higher biological value protein.

Fat content of the products showed marginal differences ($p > 0.05$), the values ranged between 2.86 ± 0.01 to 3.80 ± 0.00 g/100 g. Incorporation of fish flour resulted in lowering of fiber content. Product FMCS1 has the highest fiber content (5.34 ± 0.02 g/100 g), next to control product, of 5.81 ± 0.00 g/100 g, which has the lowest amount

(5%) of FMC and high rice and corn flour content, whereas, the product with highest amount of FMC (30%) and relatively lower amount of cereal flour blend resulted in low fiber content. Similarly, carbohydrate content of snacks increased with incorporation of high carbohydrate sources like rice and corn flour. Summarized results are tabulated in Table 3.

Generally extruded snacks are considered as energy product due to substantial amount of carbohydrate (Carvalho 2012). Further, calorific value increases since the snacks are flavored with fat or oil, as a fixer of seasonings and salt (Capriles et al. 2009). Calorific value of the FMC based-snacks ranged between 338.88 ± 0.22 and 354.56 ± 14 kcal/100 g. Samples of control snack presented calorific value of 350.20 kcal/100 g, lipids 2.36 ± 0.01 g/100 g, protein rates 7.20 ± 0.01 g/100 g and carbohydrate rates 75.02 ± 0.02 g/100 g, the snacks with fish meals improved the protein content and decreased the overall calorific value of the products. Thus, the products are having improved nutritional value when compared to control snack. Calcium and phosphorus content of the developed snacks were determined which gave quite satisfactory results.

Calcium and phosphorus contents in snacks had significant differences ($p < 0.05$) with increasing FMC incorporation (Table 3). Snacks with 30% FMC showed higher levels of Calcium when compared to control.

Snacks with smoked tilapia carcass meals presented amounts of 0.55 g/100 g, 0.34 g/100 g and 9.08 mg/100 g respectively for calcium, phosphorus and iron (Justen et al. 2011). Calcium and phosphorus content were almost similar to those reported in current study for snacks with 15–20% FMC. Inclusion of fish meals proved to be effective in enriching corn snacks with minerals especially with calcium and phosphorus.

Sensory studies

The panels of semi-trained people consisting of twenty members were given the extruded snack food samples for evaluation of organoleptic characteristics viz. appearance, flavor, taste, texture, and overall acceptability. The average score recorded by the panelist was presented (Fig. 2b). The scores of sensory evaluation showed that all the extruded products prepared from rice, corn and fish meal flours were within the acceptable range, while the extruded product prepared from rice, corn, and fish flour sample FMCS3 had significantly better appearance (7.80 ± 0.20), flavor (7.15 ± 0.05) texture (7.55 ± 0.35), taste (7.45 ± 0.25) and overall acceptability (7.88 ± 0.30). It was revealed from the scores of the overall acceptability that the 15% FMC, and 25.5% rice flour and 59.5% corn flour can be successfully mixed to produce a better acceptable product.

Table 4 Microbial study of FMC incorporated snacks

	Control	FMCS1	FMCS 2	FMCS3	FMCS4	FMCS5	FMCS6
<i>Total plant count</i>							
7 days	0	0	0	0	1×10^3 CFU/g	1×10^3 CFU/g	0
28 days	1×10^3 CFU/g	1×10^3 CFU/g	1×10^3 CFU/g	2×10^3 CFU/g	$> 1 \times 10^3$ CFU/g	3×10^3 CFU/g	1×10^3 CFU/g
90 days	$> 1 \times 10^3$ CFU/g	$> 1 \times 10^3$ CFU/g	1×10^3 CFU/g	2×10^3 CFU/g	$> 1 \times 10^3$ CFU/g	1×10^3 CFU/g	2×10^3 CFU/g
120 days	$> 3 \times 10^3$ CFU/g	$> 3 \times 10^3$ CFU/g	2×10^3 CFU/g	3×10^3 CFU/g	$> 3 \times 10^3$ CFU/g	2×10^3 CFU/g	3×10^3 CFU/g
<i>Fungus and mould</i>							
7 days	0	0	0	0	0	0	0
28 days	1×10^3 CFU/g	0	0	0	$< 1 \times 10^3$ CFU/g	$< 1 \times 10^3$ CFU/g	1×10^3 CFU/g
90 days	$> 1 \times 10^3$ CFU/g	$> 1 \times 10^3$ CFU/g	$> 1 \times 10^3$ CFU/g	2×10^3 CFU/g	$> 1 \times 10^3$ CFU/g	1×10^3 CFU/g	2×10^3 CFU/g
120 days	$> 3 \times 10^3$ CFU/g	$> 3 \times 10^3$ CFU/g	$> 2 \times 10^3$ CFU/g	3×10^3 CFU/g	$< 3 \times 10^3$ CFU/g	2×10^3 CFU/g	3×10^3 CFU/g
<i>E. coli</i>							
7–120 days	Absent	Absent	Absent	Absent	Absent	Absent	Absent

FMCS1 fish meal concentrate based snack with 5% FMC, *FMCS2* fish meal concentrate based snack with 10% FMC, *FMCS3* fish meal concentrate based snack with 15% FMC, *FMCS4* fish meal concentrate based snack with 20% FMC, *FMCS5* fish meal concentrate based snack with 25% FMC, *FMCS6* fish meal concentrate based snack with 30% FMC, *CS* control snack

Justen et al. (2011) reported that the addition of up to 12% of flavored tilapia meal did not affect sensory parameters (aroma, flavor, appearance, texture, color and overall acceptance) of extruded corn snacks, obtaining grades between 6.92 and 8.15 for all attributes.

Microbial study

The microbiological analyses of extruded snacks indicated absence of coliforms at 35 °C storage temperature for 7–120 days (Table 4), thereby indicating that the snacks are suitable for human consumption, and that they follow the microbiological standards required by law. The Total plate count for viable bacteria was found to be low up to 7 days of storage. However, rise in FMC content in snacks harbored more number of colony formation compared to snacks containing lower amount of FMC.

Conclusion

This study has presented the data on physicochemical properties in terms of crude protein, crude fat, total ash, mineral content, moisture and carbohydrates content of Bombay duck fish meal concentrate. The present study indicated Bombay duck fish meal have significant amount of protein as well as calcium, iron and phosphorous contents. The extruded fish snacks, which are the outcome of

this study showed good functional properties in terms of expansion ratio and are highly proteinaceous. The snacks fetched acceptable sensory scores in terms of organoleptic parameters as well. Overall, the products are well accepted in terms of taste, texture and nutritional properties. Fish meal concentrate like that from low cost Bombay duck fish may potentially serve as a good source of protein. Therefore, this protein source could be used as protein supplements and functional ingredients in human diets. This fish meal could be an economic and alternative protein source to alleviate protein malnutrition in developing countries.

All participants involved in sensory evaluation of extruded products provided consent prior to enrolment in the study.

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Compliance with ethical standards

Conflict of interest The authors disclose no conflict of interest in publishing this paper. There is no body to contradict this manuscript.

Ethical approval In the absence of concerned Ethical Committee, the study was undertaken after the approval by the Ph.D. committee

of School of Community Science and Technology, Indian Institute of Engineering Science and Technology, Shibpur consisting of members (Prof. Amit Kumar Das, Dean, Academic Affairs, Indian Institute of Engineering Science and Technology (IEST), Shibpur, Prof. Partha Sarathy Roy, Head, School of Community Science and Technology, IEST, Shibpur, Prof. N. R. Bandyopadhyay, School of Material Science and Engineering, IEST, Shibpur, Prof. B. K. Ghorai, Department of Chemistry IEST, Shibpur, Dr. P. Biswas, Department of Chemistry, IEST, Shibpur and Prof. P. Chattopadhyay, former Professor, Dept. of Food Technology and Biochemical Engineering, Jadavpur University). The study did not involve animal feeding and subsequent sacrifice of the animal.

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