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RESEARCH ARTICLE

OPTIMIZATION AND PRODUCTION OF EDIBLE FISH PROTEIN POWDER OF BIGEYE SCAD (*Selar crumenophthalmus*) FROM ERITREA RED SEA WATERS: PHYSIOCHEMICAL AND MICROBIOLOGICAL CHARACTERISTICS OF FRESH BIGEYE SCAD AND SHORT HEAD ANCHOVY (*Stolephorus heterobolus*).

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Abstract

Fish consumption in Eritrea is estimated at 0.5-1kg per person per year, which is very low compared to the maximum sustainable resource of the country, moreover small sized fish are used in animal feed which have great impacts on the effective utilization of the resources to alleviate malnutrtion, as one of the major problems faced. The raw material freshness, physiochemical and microbial characteristics are determining factors of edible fish protein powder (FPP). The aim of this experiment was to produce edible (FPP) from dried bigeyescad and to determine physiochemical and microbial characteristics of fresh bigeyescad, short head anchovy and edible FPP, whereby to utilize effectively and efficiently small sized pelagic fish. Bigeye scad fish were caught around the Dahlak Archipelago Islands of Eritrea Red Sea waters and short head anchovy from around Assab area by purse-seines. Fish samples were iced and transported to the fish processing laboratory of Marince Food and Biotechnology Department and identified using FAO species identification sheets, then cleaned and dried in an oven at 70 ^{o}C for 12-14 h. Morphometric and physical characteristic of the fresh fish samples of whole fish were carried out. Chemical composition of fresh samples and edible FPP was done Offical Methods of Analytical Chemistry (AOAC). Edible FPP was optimized at 15 minute cooking time, five times squeeze-pressing and washing and 12 h drying time. Minerals in all samples were determined by Alvin and Gardner method through Atomic Absorption Spectrophotometer. Quality criteria, such as free fatty acied (FFA), peroxide value (PV), thiobarbutric acid reactive substance (TBARS), pH and p-anisidine value (PAV) of fresh and edible FPP were investigated. Microbiological quality was determined by International Standards Organization methods (ISO). Edible FPP contained 82.24% protein, 7.10% moisture, 7.64% ash, 0.81% crude fiber, 4.18% FFA, 9.70 meq of 0_2/kg of fat PV, 10.78 malonaldehyde mg/kg TBARS,

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whereas Fresh bigeye scad 15.61% and short head anchovy 13.98% protein respectively. Edible FPP was rich in mineral contents, good in quality of chemical, biochemical and safe microbial parameters. Therefore, edible FPP can maximize the utilization of small pelagic fish and provide protein and mineral rich food for consumers as it had high protein and mineral contents.

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Introduction:-

Fish has been widely used as an excellent source of animal protein and other nutrients. It functions to prevent human beings from variety of diseases all over the world. Eritrean Red Sea possesses diverse marine resources. The Maximum Sustainable Yield of marine fish resource is estimated 80,000 metric tons per year and small pelagic has been estimated around 50,000 metric tons per year (Araya & Krishnan, 2012). Fish represent a valuable source of proteins and nutrients, and its consumption is high in many developed countries (Kawarazuka and Bene, 2011). Fish consumption is estimated at 0.5-1kg per person per year, which is very low compared to the maximum sustainable resource of the country, moreover small sized fish are used in animal feed which have great impacts on the effective utilization of the resources to alleviate malnutrition, as one of the major problems faced all over the world andparticularly prominent in the developing countries (IFAD, 2010; Abraha *et al*., 2017).

Small pelagic fish like bigeye scad, anchovy, sardine and mackerel are segregated separately and considered low market value (Abraha *et al*., 2017). The major problem of these fish is their size, difficult to process as time is considered. Some research has been done on low value marine fish to utilize as fish protein concentrate, fish surimi and fish powder (Gopakumar, 1997; Chattopadhyay *et al*., 2004). Many studies present technologicalalternatives and their economic feasibility that allow the processing of the small pelagic fishinto commercially attractive products for human consumption that might contribute to the alleviation of the food security problem in most developed and developing countries.

Edible fish protein powder is the first work which is done in Eritrea from small pelagic fish. FPP is adried and stable fish product, intended for human consumption, in which the protein is more concentrated than in the original fish flesh (Chattopadyay *et al*., 2004). Production of edible fish powder could be one way to solve the problems, enhance food security in the country and meet consumers demand of getting ready to use convince fish products with high nutritional values. Likewise, fish consumption could be increased if the underutilized fish resource brought in to human food chain providing as edible fish protein powder. The present work was taken up to produce edible fish protein powder from bigeyescad and to determine physiochemical and microbial characteristics of fresh bigeyescad, short head anchovy and edible FPP.

Materials and Methods:-

Fish sample collection:-

Bigeyescad (*Selar crumenophthalmus*, Bloch, 1793) were caught around the Dahlak Archipelago Islands of Eritrea Red Sea waters and short head anchovy (*Stolephorus heterolobus*, Bloch, 1793) from around Assab area by purse seines. The freshly caught fish were immediately iced after harvest on board in the ratio of 1:1 and after landing quickly transported to the fish processing laboratory of Marine Food and Biotechnology Department at Massawa College of Marine Science and Technology. Fish samples were identified using FAO species identification sheets for fishery purpose (Whitehead *et al*., 1988; Smith-Vaniz, 1983) with the collaboration of Marine Biology and Fisheries Department.

The morphometric and physical characteristic of the fresh fish samples, such as measuring total length, standard length and total weight of whole fish (Table 1) were determined. A measuring scale-ruler was used to measure length and weighing balance for weight measurement.

Equipment's and chemicals used:-

Boiling dish was used for cooking fish pieces with 23.9cm diameter and 14.5cm height, 2 litre working volume. Muslin cloth was a cotton fabric for squeezing and pressing fish meat after boiling and cooling that has around 1.00 mm mesh diameter. Drying oven industrial (Nessler electronics-scientific laboratory instruments, India) used for drying fish solid mass after squeeze-pressed. Warring blender (Warring laboratory blender food grade) used to

ground dried fish. Test sieves of A.S.T.M.E, of Geologists Syndicate PVT (Calcutta, India) were used in which the sieves standard number and opening size (mesh diameter) is No. 10 (2.00mm), No.18 (1.00mm), No.20 (850µm) , No. 35 (500 µm), No. 60 (250µm), and No. 100 (150µm).

Optimization of Edible fish protein powder Processing:-

Edible fish protein powder processing method was first optimized as per method described (Chattopadhyay *et al*., 2004) with some modifications. To optimize fish protein powder, the following factors were assumed: timing of heat treatment cooking (5min, 10, 15), number of squeezing-pressing and washing with warm water (3 times, 5 times), and drying time at 70 ^{o}C (6 h, 12 h, 18 h). Based on the trail results on yield, color, and amount of unrefined coarse particles left; the combination of fifteen minute cooking time, five times squeeze-pressing and washing, and twelve hours drying time were selected and adopted for further FPP production.

The samples were processed at fish processing technology laboratory of Marine Food and Biotechnology Department. Fresh samples were weighed, and then head and viscera were removed. The beheaded and gutted of fish samples was washed thoroughly in running tap water to remove blood, sand, slime and other extraneous matter. The washed mass was immersed into in stainless steel cylindrical cooker dish (1:2) with sufficient quantity of pre-boiled potable water to completely immerse the fish and cooked in boiling water for 15 minute under frequent agitation till the whole mass is completely disintegrated. Then, the slurry was allowed to settle by cooling so that the oil floats up the oil water emulsion is then decanted off by tilting the vessel. then operation was repeated once more.

The solid mass was transferred into a clean muslin/cheese cloth and it was squeezed and pressed manually by two persons twisting of the muslin cloth in opposite direction till draining stops. The pressed mass was fragmented and washed with running warm water. It was again squeezed and pressed. The manual hand squeezing, pressing and washing of the boiled mass while in muslin/cheese cloth was repeated five times to remove fat and the maximum amount of water. Thereafter, the pressed mass was put in aluminum trays and fragmented into smaller particles size to increase drying rate using clean or gloved hands and was evenly distributed for uniform thickness. It was dried on aluminum trays in a hot air oven drier at temperature of 70±3^{o}C for 12-14 h to a constant weight obtained approximately a final moisture level of 5% and below. The oven dried mass while it was hot, it was immediately pulverized in a warring blender at speed 2 for 90 seconds to a fine powder. The powder was sieved in a gravity type sieving of manual hand shaking and pulverized powder passed through several fitted sieves of mesh pore size (diameter) of 2mm, 1mm, 850µm, 500µm, 250µm and 150µm to have a 150-225µm. Oversized produce was pulverized once again after the first sieving step at speed 2 for 120 seconds. Finally, finer size of edible fish protein powder was obtained between 150-225µm particle sizes. Powder was packed in 100 g in 200 gauge low-density polyethylene (LDPE) plastic bags and repacked in brown paper sachets and stored at room temperature of Massawa for farther analyses.

Physiochemical analysis:-

All the physiochemical analysis of fresh samples was carried at the laboratory of Department of Food Science and Technology, Jommo-kenyatta University of Agriculture and Technology-JKUAT, Nairobi, Kenya. Proper packing system and cold chain system was followed for transporting samples.

Proximate analyses:-

Crude protein, moisture, fat, and ash were determined using conventional method of AOAC (1990). Crude protein content was determined using the semi-micro Kjeldahl method. Moisture content was determined by drying in hot air oven for 4 h at 105 °C until constant weight was achieved. Crude fat content was measured by the Soxhlet method. Ash content was determined by incineration method, which sample was combusted in muffle furnace at 550-600 °C for 2 h.

Crude fibre was determined by Hennenberg- Stohmann method-978.10 (AOAC, 1995), 2g of sample was weighed and transferred to a 250 ml volumetric flask. 200 ml of boiled 1.25% H_2SO_4 was added and boiled for 30 minutes. The digest was filtered over a fibre glass and washed. The fibre glass was put in a conical flask and 200 ml of 1.25% NaOH was added and solution boiled for 30 minutes. The solution was filtered and washed with 1% HCl and then boiled in water. The filter was then washed with diethyl ether. The fibre glass and samples were then transferred on a crucible. After oven dried was cooled and then weighed (W_1). To burn the fibre, the crucible with the sample was incinerated at 550^{0}C; it was then cooled and weighed (W_2). Percentage of crude fibre was expressed as % crude fibre equal to ((W_1-W_2) x100)/ (Sample Weight).

Minerals, pH, salt content and color analyses:-
Minerals concentrations such as Calcium, Magnesium, Zinc, Iron, and Cadmium were measured using Alvin and Gardner (1986) method through Atomic Absorption Spectrophotometer (Shimadzu 6300 AAS AA/AE, Europe). Agustini research (2001) method was followed to measure pH of fresh fish that was analysed using digital pH meter (HI8519N-model Hanna Instruments Inc, Woonsocket, RI, USA). Color of fresh samples and edible fish protein powder was measured by placing them in a test tube (25 mm in diameter) which was read in a Minolta CR-200b Chroma Meter (Minolta Camera Co. LTD.Osaka, Japan) in Lab* measuring mode (CIE, 1976) with CIE Illuminant C. The Color was measured three times turning the test tube 120° between measurements. Estimated results were given as lightness (L*), redness (a*) and yellowness (b*). Salt content as sodium chloride was estimated by Mohr's method as described in Sheen and Kahler (1938).

Determination of lipid oxidation:-
Analysis of fish oxidation was carried out after extraction of lipids from fresh samples. The total lipid was estimated bythe method of Bligh and Dyer (1959). The extracted Lipid was used to estimate Free Fatty Acid (FFA) following AOCS-Ca-5a-40 (AOCS, 1998) titration method and free fatty acid results were expressed as % of oleic acid of total lipid. Peroxide value (PV) was determined by iodimetric titration method of AOCS-Cd-8-53(AOCS, 1998) and expressed as milli-equivalent of oxygen per kilo gram of lipid. Thiobarbituric acid reactive substances (TBARS) was determined using spectrophotometer at 532 nm according to the AOCS (1998) method and TBA/TBARS values were expressed as milligram of malonaldehyde (MDA, Malondialdehyde) equivalents per kilogram offish meat. Estimation of p-anisidine value (p-AV) of lipid extracted from fresh fish was done using IUPAC- 2504 Method (IUPAC, 1987) and value was expressed in anisidine numbers.

Microbiological analysis:-
All the microbiological analysis of samples were conducted in the Quality Control Laboratory (QCL), Ministry of Marine Resources (MMR) at Massawa, Eritrea. It was done using International Standards Organization methods (ISO). Preparation of all samples, initial suspension and decimal dilutions was based on ISO 6887-4:2003. The microbiological analysis on enumeration of microorganisms for Total Plate Count (TPC) using pour plate technique, spread plate for fungal and halophiles count was done using standard methods (ISO 4833:2003) at 30 ^{o}C.

In Total plate count (TPC), 22g of fish sample was homogenized using 198ml peptone water solution in stomacher bag and then ten-fold serial dilution was prepared. From initial suspension (10^{-1}), 1ml transferred to serial dilution of test tube with 9ml peptone water, and parallel with this from all dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}), 1ml was transferred to a duplicate sterile Petri-dishes with molten plate count agar. The solidified plates were inverted and incubated at 30 ^{o}C for 72 h. Finally the number of colonies were counted and multiplied by dilution factor to calculate the total colonies forming units per gram of sample (cfu/g).

Halophilic bacterial count was done with slight modification of standard methods (ISO 4833:2003). Halophilic bacterial count was determined using 0.85% sodium chloride solution as diluent. Plating was done onto plate count agar with 10% salt by spread plate technique. The colonies developed in the planter were counted and expressed as number of colony forming units/g of sample (cfu/g).

Total fungal count (TFC) was carried out with slight modification of standard methods (ISO 4833:2003), 10 g of fish sample was weighed aseptically and homogenized with 90 ml of physiological saline solution. Appropriate dilutions were made from the 9.0 ml physiological saline and plated onto Dicorosal Rose Bengal Agar containing Chloramphenicol (DRBC) plate. The plates were incubated at 30^{o}C for 3-5 days and TFC were enumerated and recorded as cfu/g.

ISO (4831:2006) was used to enumerate the total coliforms count at 44 ^{o}C using multiple tube technique**.** From 22:198 ratio of fish to peptone water dilution homogenate, 10 ml, 1ml, and 0.1ml sample (equivalent to 1 mL of serially diluted 1:10, 1:100 and 1:1000) was transferred into 9 mL sterilized Lauryl Sulphate Tryptose (LST) broth in triplicate (3 test tubes). For each dilution, the tubes were incubated at 37^{0}C for 48 ±2 h to evaluate gas formation. Lauryl Sulphate Tryptose (LST) broth was used as a pre-enrichment media. After primary incubation, one (0.3 mm) loopful of positive tubes (gas formation by the action of the coliform bacteria in fermenting lactose medium tubes) was transferred to Brilliant Green Lactose Bile (BGLB) broth, further incubation was done at 37 ^{o}C for 48±2 h for total coliforms count. Inverted Durham Fermentation Tube was added into test tubes before the addition of BGLB broth to allow easy identification of gas production. Then the number of tubes with positive gas production were

counted. Most probable Number (MPN) of Coliform bacteria per gram sample was calculated from MPN table based on the number of tubes of BGLB broth producing gas at the end of incubation period.

Multiple tube technique was also followed to enumerate β-glucuronidase-positive *Escherichia coli* (*E.coli*) (ISO 16649-3:2001)**.** From 22:198 ratio of fish to peptone water dilution homogenate, 10ml, 1ml, and 0.1ml sample (equivalent to 1 mL of serially diluted 1:10, 1:100 and 1:1000) was transferred into triplicate (3 test tubes) with 9 mL sterilized Mineral modified glutamate medium (MMGM). For each dilution, the tubes were incubated at 37°C for 24 ±2 h to acid production. After primary incubation in MMGM pre-enrichment media, one (0.3 mm) loopful of positive tubes was inoculated by streaking on perti dishes containing Tryptone-Bile-Glucuronic Agar (TBX), further incubated for 20-24 h at 44±1^{o}C and then the petri dishes with positive result was observed. MPN of *E.coli*per gram sample was calculated from MPN table based on the number of positive results (look a typical blue colonies).

Enumeration of coagulase-positive *Staphylococcus aureus* (*S.aureus*) by spread plate technique, ISO (6888-1:2003) was followed. From 22:198 ratio of fish to peptone water dilution homogenate, 1ml transferred to serial dilution of test tube with 9ml peptone water, and parallel with this from all dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}), 0.1ml was transferred to a duplicate sterile petridishes containing pre-solidified Baird-Parker agar. The inverted plates incubated at 37±1^{o}C for 24± 2 h. typical black colonies with white margin (halo) and a clear zone around colonies on Baird parker agar were enumerated and inoculated into Brain Heart infusion (BHI) broth test tubes and after 37±1^{o}C for 24±2 h incubation, 0.1-0.3ml rabbit plasma was added to the test tubes with positive growth and incubated at 37±1^{o}C between 4-6 h. Positive tubes showed full coagulation and clots in the liquid BHI were enumerated and recorded as cfu/g.

Salmonella spp. was isolated and detected using isolation technique ISO (6579:2002). 25 g of sample was homogenized and enriched in 225 ml Buffered peptone water at 37 °C for 24 h. Selective enrichment of Salmonella was carried out by transferring 1ml to test tubes containing Muller-Kauffmann-tetrathionate/novobiocin (MKTTn) broth and Rappaport-Vassiliadis-medium with soya (RVS) brothin thermostatically controlled water bath. From each of these enriched cultures was streaked on Xylose Lysine Deoxycholate Agar (XLDA) and Brilliant Green Agar (BGA) incubated at 37±1^{o}C for 24±3h. Typical Salmonella exhibit pink colonies with or without black centers in XLDA and pink color in BGA. Biochemically confirmed in TSI Agar (Triple sugar Iron), Urea Agar, L-Lysine Decarboxylation Medium, Detection of β-galactosidase, Voges-Proskauer Reaction, Indole Reaction, Kovac's Reagent, H_2S, Physiological salineincubated at37+/-1^{o}C for 24±3h. If biochemical tests shows no positive *Salmonella s*pp isolatesand confirmation in antisera is negative (no agglutination). The final result is absence of *Salmonela* spp in 25 g of fresh meat.

Vibrio parahaemolyticus was isolated and detected according to ISO (8914-1990). 25 g of sample was homogenized and enriched in 225 ml of alkaline peptone water (APW) with 3% sodium chloride at 37±1^{o}C for 24-48±3h. Selective isolation of *Vibrio* was carried out by transferring 1ml into thiosulphate citrate bile salt sucrose agar (TCBS) with 3% sodium chloride. Presence of *Vibrio parahaemolyticus* shows green colored colonies. Each selected colony was inoculated by streaking onto the surface of nutrient agar. Colonies from nutrient agar slant test tube used the biochemical testsuch as Sucrose, oxidase, Motility, Glucose (acid), gas formation from glucose, Lactose acid, H_2S, Aerobic and anaerobic growth, Lysine descarboxylation, Indole, B-galactosidase. If the biochemical test shows no (negative) *Vibrio parahaemolyticus*, the result is reported as absence of *Vibrio parahaemolyticus* in 25g of fresh meat.

Statistical analysis:-

All the results presented are in means of triplicate sample. Data obtained were subjected to one way analysis of variance and the levels were differentiated using Post-Hoc Tukey-Duncan's multiple range tests in Statistical Package for the Social Sciences (SPSS) software version 20. Significance was ascertained at $p < 0.05$.

Results and Discussion:-

Fresh fish samples characteristics and FPP yield:-

Bigeye scad and short head anchovy are small sized pelagic fish. The size and weight of small pelagic fish is important in the yield of the sample to utilize. Table 1 gives characteristic measurement of weight and length of both bigeye scad and short head anchovy. The average weight and length of the samples used in this study was 23g, 21g and 15cm , 13cm for bigeyescad and short head anchovy respectively, showed that the fishes were of adult size and recommended for utilization as per the yield is concerned. Result was related with Chattopadhyay *et al*. (2004), who

use silver bellies (*Leiognathus spp*) with length range from 3.8 to 8 cm to prepare edible fish powder. The total length of these species ranged from 14 cm to 16 cm. The total length of bigeyescad was 16 cm which is similar to the result found by Metillo and Aspiras Eya (2014).

Table 1:- Fresh small pelagic fish species characteristic measurements before processing

Parameters	Bigeye scad	Short head anchovy
Biggest fish Total length (cm)	16	14
Smallest fish Total length (cm)	14	12
Average Total length (cm)	15	13
Biggest fish Fork/Standard length(cm)	14.5	10.25
Smallest fish Fork/standard length (cm)	13	7
Average Fork/standard length (cm)	13.75	11
Biggest fish weight (g)	30	24
Smallest fish weight (g)	13	11
Average weight fish piece per kg (g)	23	21
Number of fish pieces per kg	26	28

The yield obtained from both samples is given in Table 2. To increase the yield, quality of FPP and produce at lower cost, optimization trail was conducted. The parameter combinations of processing of 15 mins cooking (heat treatment) in boiling water, 5 times washing and squeeze-pressing, 12-14 h dried at 70 ^{o}C, ground in warring lab blender and sieved in less than 150 micron was marginally better in yield, quality of produce and lower discards (Table 2). Thus, the optimized method was used in all samples reduction to FPP. The fresh fish samples subjected to weight reduction to FPP using combined processing of 15 min cooking in boiling water, 5 times washing and squeeze-pressing, 12-14 h dried at 70 ^{o}C, ground in warring lab blender and sieved in less than 150 micron. It showed different values of yield among two species of small pelagic fish sampled (Table 2).The FPP yield was 8.35% in bigeye scad and 6.9% in Short head anchovy. The reason for low yield of these species may be the body muscle to bone composition ratio is low and higher water content of wet fish. This study FPP yield is comparable. To make 1kg FPP requires approximately 5–10 kg fresh fish (Shaviklo, 2015). Prices of underutilized fish (small pelagic species) are relatively low because of the availability of such raw material in bulk at a relatively cheap price. The production of FPP from small pelagic species is attractive and can contributing in food security.

Table 2:-Yield obtained from bigeye scad and short head anchovy after processing

Parameters	Bigeye scad	Short head anchovy
Initial Weight of sample (g)	7800	550
Weight after behead and deveining (g)	7610	260
Boiling time (mins)	15	15
Number of squeezing	5 times	5 times
fine powder weight (<150 micron) (g)	651.6	37.882
Yield (%)	8.35	6.9

Fish can be considered as a potential, cost effective source to enhance chemical composition intakes and it is a capable as a complementary food for consumers particularly under nourished children (Kawarazuka and Bene, 2011). The chemical composition of raw bigeyescad, short head anchovy and edible fish protein powder made out of bigeyescad are shown in Table 3. The crude protein, crude fat, moisture, ash and crude fiber for bigeyescad were 15.61±1.33%, 4.77±%, 75.55±0.5%, 75.55±1.03%, 1.91±0.29%, 0.40±0.17% respectively. Similar composition were reported for mackerel (*Rastrelliger kanagurta*) with a protein 16.75±0.55%, fat 5.03±0.87%, moisture 75.2±0.5% (sumi *et al*., 2016) as well as by Abbey *et al*. (2017a) for flying gurnard (*Dactylopterus volitans*) and Dogonda *et al*. (2014), who studied the proximate composition of *Rastrineobola argentea* (Dagaa). The proximate composition in short head anchovy was 13.98±1.16 protein, 3.51±0.57 fat, 79.63±1.07 moisture, 1.94±0.18 ash and 0.26±0.02 crude fiber. Similar results were found by Karakoltsidis *et al*. (1995) for the fish family of Clupeidae (pilchards) and by Abbey *et al*. (2017b) for burrito (*Bachydeuterus auritus*). Result found in chemical composition of fresh fishes were also in agreement with Suseno *et al*. (2014), who studied chemical composition of spotted sardinella (*Amblygaster sirm*) and gold-strip sardinella (*Sardinella gibbosa*). And results were also in accordance with earlier finding by Chattopadhyay *et al*. (2004) and Barman *et al*. (2014). Fish protein powder obtained from

bigeye scad was 82.24% crude protein, 6.98% crude fat, 7.10% moisture content, 7.64% ash and 0.81% crude fiber. This study is in close approximation to that reported by Sathivel *et al*. (2004) studied functional, nutritional, and rheological properties of protein powders from arrowtooth flounder and their application in mayonnaise. The protein content edible FPP found in the present study was higher than protein content (62.55%) found by Chattopadyay *et al*. (2004) and Barman *et al*. (2014). The difference might be chemical composition of fish and methods used. Moisture content of FPP was below the range most researchers found. Moisture content between 5% and 10% is quite normal (Burt *et al*., 1992). Protein content was significantly different ($p<0.05$) among bigeye scad (15.61±1.33), short head anchovy (13.98±1.16) and FPP (82.24±1.98), likewise the other constituents. Previous studies have reported that fish can be grouped into four categories according to their fat contents: lean fish (<2), low fat (2-4), medium fat (4-8) and high fat (>8) (Ogonda *et al*., 2014). According to the result found in this study, bigeye scad and short head anchovy are medium fatty fish.

The result of the present study revealed that the FPP was with ample nutritional composition which might have to play a great role in human health. Consumption of fish protein powder produced from such types of fish incorporation with basic food could improve nutritional value of the food and biological value of the diet, particularly for children who have difficulties in digesting carbohydrate. Sánchez and Gallo **(**2009) reported that these small pelagic fishes such as, scad, anchovy and other species, are an excellent source of high-quality protein, lysine and methionine can be mentioned which makes these species a suitable complement to carbohydrate rich diets where protein sources are insufficient. The protein content of small pelagic fish and FPP are above the levels of 15-20% and 72-83% of fish body weight for fresh fish and dry powder reported for small pelagic fish (Ogonda, 2014; Abbey *et al*., 2017b). This implies that it can be used in food supplements.

Table 3:- Chemical composition of fresh bigeye scad, short head anchovy and edible fish protein powder (FPP) made out of bigeye scad

Constituents (%)	Fresh bigeye scad	Fish protein powder	Fresh short head anchovy
Crude Protein	15.61 ± 1.33^{b}	82.24 ± 1.98^{c}	13.98 ± 1.16^{d}
Crude Fat	4.77 ± 0.50^{d}	6.98 ± 3.84^{c}	3.51 ± 0.57^{a}
Moisture	75.55 ± 1.03^{a}	7.10 ± 0.73^{b}	79.63 ± 1.07^{c}
Crude Ash	1.91 ± 0.29^{a}	7.64 ± 0.05^{c}	1.94 ± 0.18^{b}
Crude Fiber	0.40 ± 0.17^{b}	0.81 ± 0.12^{a}	0.26 ± 0.02^{c}

Values are Mean ±SD (standard deviation), Values in the same row not sharing the same superscript are significantly different at $p<0.05$.

The free fatty acid, peroxide value, thiobarbutric reactive substance, p-anisidine value and salt are shown in Table 4. The FFA values of each samples was significantly different likewise, the other quality criteria. The FFA value was lower in fresh samples of bigeye scad and short head anchovy compared to edible fish protein powder. This could be due to their difference in their fat and protein contents. The relationship between FFA release and loss of freshness was reported by Ozogul *et al*. (2005), who studied the freshness of European ell (*Anguilla anguilla*).

Fish and fish products may show off odour, taste rancid and no nutritional values when PV, FFA and TBARS are above 20 mill moles of oxygen per kg of fat (Abraha *et al*., 2017b), 10-20 millimole of malonaldehyde/kg of fish lipid (Abbey *et al*., 2017a). Nishimoto *et al*. (1985) reported that, the early development of rancidity is indicated by the presence of peroxide value. Thiobarbituric acid is widely used indicator for the assessment of degree of secondary lipid oxidation in fish and fish products (Nishimoto *et al*., 1985). However, all the quality criteria values found in this study were below the acceptable limit and no rancidity was observed. The reason could be due to low water content, no mould and enzymes activities (in case of FPP) and the low values are indications of the level of freshness of the studied fish species and that they may not have undergone any major quality deterioration in terms of lipid oxidation and its associated reactions. This result is in agreement with the findings of previous study (Chattopadhyay *et al*., 2004; Immaculate *et al*., 2012). pH is also an important index for evaluating the quality of fish (Okeyo *et al*., 2009). Edible FPP had pH 6.40±0.01 for which reveals a good quality product. p-anisidine value is another measurement of the extent of oxidative deterioration. It was determined by spectrophotometric assay (at wavelength of 350 nm) of aldehydes and ketones in the lipid by reaction with p-anisidine solution. The results of raw fish and edible fish protein powder were below the acceptable limit (Table 4). Sikorski (2003) suggested that PAV approaching 10 indicates that considerable oxidation has occurred and the accumulation of rancid compounds.

The results of biochemical qualities reveal that the fresh bigeye scad, fresh anchovy and edible FPP were good in quality and nutrition.

Table 4:- Quality criteria of fresh bigeye scad and Short head anchovy and edible fish protein powder (FPP) produced from bigeyescad.

Quality criteria	Fresh bigeye scad	Fish protein powder	Fresh short head anchovy
FFA (% of oleic acid)	2.13±0.10[b]	4.18±0.51[a]	1.96±0.30[c]
PV (meq of O_2/kg of fat)	5.38±0.56[c]	9.70±1.76[b]	10.40±0.16[a]
TBARS (mg, malonaldehyde/kg)	2.14±0.13[a]	10.78±0.56[c]	2.26±0.42[b]
PAV (%)	3.87±0.18[b]	5.28±0.34[a]	2.59±0.55[c]
pH	7.36±0.04[c]	6.40±0.01[b]	7.27±0.01[a]
Salt (%)	0.90±0.13[c]	0.11±0.01[a]	0.74±0.15[b]

Values are Mean ±SD (standard deviation), values with the different superscription at the same raw are significant different at $p<0.05$

Small pelagic fish are rich in mineral content in addition to chemical composition (Smichi*et al*., 2016). They contain high amount of magnesium, lead, potassium, iron, phosphorus and calcium which is significant for the health of consumers (Sánchez and Gallo, 2009).

Table 5 shows the mineral content of fresh, short head anchovy and FPP made out of bigeye scad. Edible fish protein powder was rich in mineral contents (Table 5). Calcium content was 193.44±10.25 for bigeyescad, 1620.94±25.76 for FPP and 415.46±45.95for short head anchovy, whereas iron content 4.09±0.07for bigeye scad, 15.64±1.06for FPP and 3.96±0.41for short head anchov. The results were similar with recent published article by Abbey *et al*. (2017a and 2017b), who studied Proximate and biochemical characterization of burrito (*Bachydeuterus auritus*) and flying gurnard (*Dactylopterus volitans*) and Nutrient content of fish powder from low value fish and fish byproducts. Similar results were also reported by Chattopadhyay *et al*. (2004), Barman *et al*. (2014) and Smichi *et al*. (2016). In fresh condition bigeye scad had low mineral content compared to FPP. FPP produced from bigeyescad had high amount of calcium (1620.94±25.76) and magnesium (463.33±81.85) content. This indicated that the mineral content was higher in FPP than in fresh condition, this could be attributed due to the reduction of water content from the FPP product and from the bones of the fish. There was significant difference ($p<0.05$) in mineral content among fresh bigeyescad, short head anchovy and FPP. This could be due to their difference in their water content 75.55±1.03, 79.63±1.07 and 7.10±0.73, respectively. Short head anchovy was higher in calcium content compared to bigeyescad. Anchovy have highcontent of minerals (K, Fe, P, Ca, I) and vitamins (A and D), as well as valuablesource of omega-3 fatty acids (EPA and DHA) that are essential, especially for pregnant and nursing women (Sanchez and Gallo, 2009).

The mineral contents of the two species though quite more were also below the values of 580mg/100 g of sample reported for other small pelagic fish (Sidwell, 1981). Although the magnesium content of the bigeyescad, FPP and short head anchovy were significantly different from each other, they are within a wider range 0.8mg/100g up to 373mg/100g reported for other small pelagic (Sidwell, 1981; Teeny *et al*., 1984).

Table 5:- Mineral content of fresh and edible fish protein powder (FPP) of Bigeye scad and Short head anchovy

Constituents(mg/kg)	Fresh bigeye scad	Fish protein powder	Short head anchovy
Ca	193.44±10.25[a]	1620.94±25.76[b]	415.46±45.95[c]
Zn	1.82±0.10[a]	10.77±0.07[b]	2.28±0.12[c]
Fe	4.09±0.07[c]	15.64±1.06[a]	3.96±0.41[b]
Cd	0.04±0.00[b]	0.05±0.03[a]	0.03±0.01[c]
Mg	64.54±0.78[a]	463.33±81.85[b]	57.77±1.14[c]

Values are Mean ±SD (standard deviation), Values in the same row not sharing the same superscript are significantly different at $p<0.05$

Color for fresh bigeyescad, fresh short head anchovy and edible fish protein powder prepared from bigeyescad was measured. Color expressed as lightness (L*), redness (a*) and yellowness (b*). Color attributes are the most

determinant factors of physical characteristics of fish and fish products. The data presented at Table 6 are color attributes of raw samples and FPP. Color values of the raw bigeye scad were very good and acceptable. Stillings *et al*. (1971) reported that color attributes of raw material can influence the color of finished products. FPP produced from bigeyescad (L*, 58.13±3.89) possessed the highest lightness (L*, 70.13±0.57). The redness (a*) and yellowness (b*) values of FPP were 0.33±0.15 and 12.93±0.21, respectively. The results of color attributes evaluation revealed that the FPP produced showed the highest lightness and lowest redness. Significant differences ($p<0.05$) were observed among the raw and edible fish protein powder in terms of lightness, redness and yellowness. This could be due to difference in their chemical composition and treatment done for FPP, squeezing and pressing play a role in reduction of fat content.

Table 6:- Color characteristics value of raw and edible fish protein powder (FPP) made out of bigeye scad and raw short head anchovy

Color value	Fresh bigeye scad	Fish protein powder	Short head anchovy
L*	58.13 ± 3.89^{c}	70.13 ± 0.57^{a}	54.23 ± 9.39^{b}
a*	4.83 ± 1.30^{b}	0.33 ± 0.15^{c}	1.00 ± 0.20^{a}
b*	11.23 ± 1.05^{a}	12.93 ± 0.21^{b}	5.53 ± 0.61^{c}

Values are Mean ±SD (standard deviation), Values in the same row not sharing the same superscript are significantly different at $p<0.05$.

The quantity of bacterial and fungal in fish and fish products serves as a universal indicator of cleanliness. evaluation of bacterial count is widely used to measure the bacterial and fungal quality of fish and fish products. Microbial analyses of fresh bigeyescad, fresh short head anchovy and FPP produced from bigeyescad are shown in Table 7. The moisture content of FPP was 5.10±0.73. Osibona *et al*. (2009) reported that moisture content below 10% is good for microbial safety of fishery products. In the present study, very low TPC fresh bigeye scad, short head anchovy and FPP ($1.1x10^{2}$cfu/g) were found. The low moisture content and hygienic condition might be attributed to low TPC. The border for total plate count (TPC) is 1×10^{5}cfu/g in the dried product **(**Relekar *et al*., 2014, Abraha *et al*., 2017). Significant difference ($p<0.05$) was observed in TPC among bigeye scad, short head anchovy and edible fish protein powder. Low moisture content and the temperature applied in drying might subject to lead in differences. The quality of both fresh and edbile fish protein powder were found to be very good as the microbial parameters were below the acceptable limit (Connell, 1975). In fresh samples <3 total coliforms, <3 *E. coli*, $1.0x10^{2}$ cfu/g *Staphylococcus aureus* for bigeyescad, <4 total coliforms, <2 *E. coli*, $1.0x10^{3}$ cfu/g *Staphylococcus aureus* for short head anchovy were found, whereas in FPP were not detected. The pathogens *E. coli, Staphylococcus aureus, Salmonella, Vibrio, parahaemolyticus,* halophilic count, total fungal count were not detected in FPP produced from bigeyescad. The FPP produced was dried in an oven at 70 ± 3^{o}C for 12-14 h which was effective to inactivate TPC and to reduced moisture content, whereby to record acceptable result, and to kill total coliforms, *E. coli,* and*Staphylococcusaureus* which were in few quantities in fresh samples. The maximum allowable number of *E.coli* in raw fish and fishery products is 20 / g (Weagant *et al*., 1995). This result is in accordance to with the earlier findings (Abbey *et al*., 2017b), who studied on Nutrient content of fish powder from low value fish and fish byproducts.

Table 7:- Microbial analyses of fresh bigeyes scad, short head anchovy and edible fish protein powder (FPP) of bigeyes scad.

Microbial parameters	Fresh bigeye scad	Fish protein powder	Short head anchovy
TPC, cfu/g	$1.6x10^{2b}$	$1.1x10^{2a}$	$1.8x10^{2c}$
Total coliforms, MPN/g	$<3^{c}$	Absent	$<4^{b}$
E.coli MPN/g	$<3^{a}$	Absent	$<2^{b}$
Staphylococcus aureus, cfu/g	$< 1.0x10^{2c}$	Absent	$< 1.0x10^{3b}$
Salmonella spp. Cfu/25g	Absent	Absent	Absent
Vibrio parahaemolyticus, cfu/25g	Absent	Absent	Absent
Halophilic count, cfu/g	Absent	Absent	Absent
Total fungal count, cfu/g	Absent	Absent	Absent

Note: Values are Mean ±SD (standard deviation), Values in the same row not sharing the same superscript are significantly different at $p<0.05$

Conclusion:-

In the present study edible fish protein powder was made out of bigeyescad. Edible FPP had rich in protein, mineral contents and attractive color. Nutritional studies showed that edible fish protein powder can be used as value added product to meet the consumer demands particularly for children and nursing mothers. It can be suggested that edible FPP could be an alternative source of protein and mineral for consumer at large to contribute in food security of the country. Hygienic handling of the product during the process especially at the time of processing, squeezing, pressing, grinding and packaging is essential to prevent external microbial contamination. Result of the present study are expected to provide somebasic information about this almost unknownproduct for further studies and further study is required to assess and utilize the commercial potential of the small pelagic fish in Eritrean Red Sea waters.

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