World Academy of Science, Engineering and Technology
International Journal of Nutrition and Food Engineering
Vol:8, No:1, 2014

Antioxidant Properties and Nutritive Values of Raw and Cooked Pool Barb (*Puntius sophore)* of Eastern Himalayas

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Abstract**—**Antioxidant properties and nutritive values of raw and cooked Pool barb, *Puntius sophore* (Hamilton-Buchanan) of Eastern Himalayas, India were determined. Antioxidant activity of the methanol extract of the raw, steamed, fried and curried Pool barb was evaluated by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging assay. In DPPH scavenging assay the IC_{50} value of the raw, steamed, fried and curried Pool barb was 1.66 micro-gram/ml, 16.09 micro-gram/ml, 8.99 micro-gram/ml, 0.59 micro-gram/ml whereas the IC_{50} of the reference ascorbic acid was 46.66miro-gram/ml. These results showed that the fish have high antioxidant activity. Protein content was found highest in raw (20.50±0.08%) and lowest in curried (18.66±0.13%). Moisture content in raw, fried and curried was 76.35±0.09, 46.27±0.14 and 57.46±0.24 respectively. Lipid content was recorded 2.46±0.14% in raw and 21.76±0.10% in curried. Ash content varied from 12.57±0.11 to 22.53±0.07%. The total amino acids varied from 36.79±0.02 and 288.43±0.12 mg/100g. Eleven essential mineral elements were found abundant in all the samples. The samples had considerable amount of Fe ranging from 152.17 to 320.39 milli-gram/100gram, Ca 902.06 to 1356.02 milli-gram/100gram, Zn 91.07 to 138.14 milli-gram/100gram, K 193.25 to 261.56 milli-gram/100gram, Mg 225.06 to 229.10 milli-gram/100gram. Ni was not detected in the curried fish. The Mg and K contents were significantly decreased in frying method; however the Fe, Cu, Ca, Co and Mn contents were increased significantly in all the cooked samples. The Mg and Na contents were significantly increased in curried sample and the Cr content was decreased significantly ($p<0.05$) in all the cooked samples.

Keywords**—**Antioxidant property, Pool barb, minerals, amino acids, proximate composition, cooking methods.

I. Introduction

INDIA is a vast country with a population of one billion, it ranks second in the world next only to China with an area of 32,87, 263 sq.km. The Eastern Himalaya is a biodiversity rich region. The diversity is attributed to the recent geological history (the collision of Indian, Chinese and Burmese plates) and the Himalayan orogeny which played an important role in the speciation and evolution of groups inhabiting mountain streams [1]. Nestling in the southern foothills of the Eastern Himalayas in this vast country is the North Eastern (NE) region of India. The NE (latitude and East longitudes) covers an area of 2,55,083 sq. Km., which accounts for 7.8% of the total landmass of India. The NE comprised of eight states-Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim and Tripura. *Puntiussophore* (Hamilton-Buchanan) (Family- Cyprinidae) is locally known as "Phabou-nga" in Manipur and commonly called as Pool barb. The cooked and fried fish of *P. sophore* is locally called as "Phabou-Nga-Thongba" and "Phabou-Nga-Ataoba" in Manipur. The vegetable like *Alocasia indica* (Schott) commonly known as Elephant's ear plant and locally known as "Yendem" in Manipur is mostly added in the curried Pool barb.

Small Indigenous freshwater Fish (SIF) are defined as fishes which grow to the size of 25-30cm in mature or adult stage of their life cycle. They inhabit in rivers and tributaries, floodplains, ponds and tanks, Lakes, streams, lowland areas, wetland and paddy fields [2]. Small Indigenous fishes traditionally occupy an unenviable position and an inseparable link in the life, livelihood, health and the general well of the rural mass, especially the poor. An antioxidant is a molecule of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate these chain reactions by being oxidized themselves. As a result, antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenol [3]. Minerals have several functions in the body, including the formation of the body structure, co-factor for enzymes and transformation of energy. They are constituents of the organic compounds such as proteins and lipids which make up muscles, organs, blood cells and other soft tissues of the body. Moreover, they are important in activation of enzyme and hormone systems. Cooking methods are important parameters for chemical composition and nutritive value of fish muscle [4]. Although fish is a good source of essential nutrients, cooking practices could cause modifications in proximate composition, fatty acids and amino acids as well as changes in solubility and nutritional quality of fish [5]. Another study indicated that cooking of fish consumed in Saudi Arabia leads to alteration in cholesterol, fat and protein content but the cholesterol content of raw and cooked fishes was not directly correlated to fat content [6].

In Manipur there are some reports on fresh and processed fishes [7], [8]. However, there is no report so far on cooking methods/cooking effects on biochemical composition of fish used in our daily life. Thus, these studies was carried out to

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determine the Antioxidant properties and nutritive values of raw and cooked small Indigenous fish Pool barb (*Puntius sophore*) of Eastern Himalayas.

II. Materials and Methods

A. Sample Collection

Fresh *Puntius sophore* was collected from Imphal and Moreh market of Manipur, Ujjan market of Guwahati, Assam, Lamphalong, Myanmar and other different markets of Eastern Himalayas. The vegetables like elephant's ear plant, tomato and pea were purchased from different market and brought to the Life Sciences Department, Manipur University.

B. Sample Preparation and Cooking

Fresh fish were not chopped or beheaded and washed with tape water several times. The vegetables (*Alocasia indica*, tomato, pea, coriander leaf) were washed and chopped. 1kg of fish was divided into four equal lots, each lot equivalent to 250g. The first lot was uncooked while the other three lots were cooked in the following methods i.e. steaming, frying and currying. Steaming was done in a pressure cooker (Hawkins pressure cooker). The frying of fish was carried out in a frying pan of 2 liter capacity at temperature 180^0C for 4 minutes. Soybean oil was used for pan-frying and its temperature during the frying process was 180^0C. Fried fish was cooked with chopped vegetables for 35min. After the cooking process, for all methods, the bones and skins of fish were not removed and from fish curry vegetables were removed. All fish in each lot were homogenized using a mortar and pestle and analyzed to determine proximate composition and mineral contents. All assays were conducted on triplicate samples of the homogenates.

C. Determination of Antioxidant Properties and DPPH Scavenging Activity

The antioxidant activity of the Small fishes extract was examined by comparing it to the activity of known antioxidants such as ascorbic acid by scavenging of DPPH radical scavenging activity. For antioxidant properties, the dried fish samples were extracted in 90% methanol solution. The free radical scavenging capacity of the extracts was determined using DPPH (1,1-diphenyl-2-picrylhydrazyl) [9]. The reaction mixture consisted of 125 µM DPPH with 5 µg/ml, 10 µg/ml, 15 µg/ml and 20 µg/ml of the fish extract. The stock solutions of 0.1mM ascorbic acid are used as reference antioxidants. After a 30 min incubation period in the dark room temperature, the absorbance was read against a blank at 517 nm. Percentage inhibition was determined by comparison with a methanol treated control group.

DPPH decolouration (%) (1-OD sample/OD control) ×100

The degree of decolouration indicates the free radical scavenging efficiency of the substances.

D. Determination of Total Free Amino Acids, Proximate Composition and Mineral Elements Contents

The total free amino acids were determined according to the [10]. The Calculation of the amount of total free amino acids was done using standard curve prepared from leucine by pipetting out 0.1-1.0ml (10-100µg range) of working standard solution. The results were express as percentage equipment of leucine. Proximate composition analysis for homogenized samples of raw and cooked fish were done in triplicate for protein, moisture, fat and ash contents. The moisture content was determined by drying the fish at an oven at 60^0C until a constant weight was obtained. Crude protein content was calculated by converting the nitrogen content determined by micro Kjeldahl's method (6.25× N). Fat was determined by using the Chloroform-methanol (2:1, v/v) solvent system. Ash content was determined by dry ashing in a furnace at 550^0C for 2 hour [11].For mineral determination, 5g of respective raw and cooked samples were digested in HNO_3[12]. The digest was quantitatively transferred to a 50 ml volumetric flask and made up to volume with distilled water. A blank digest was carried out in the same way. The elements Fe, Cu, Zn, Ca, Co, Mn, Mg, Cr, Na and K were measured by atomic absorption spectrophotometry (AAS) using a Perkin Elmer spectra Atomic Absorption spectrophotometer model 3110. The mineral concentration was expressed as mg mineral/100g fish dry weight.

III. Statistical Analysis

The data were analyzed using one-way analysis of variance (ANOVA) and the significant differences between means of experiments were determined by post hoc Duncan's multiple range test. A significance level of 0.05 was chosen. Data were analyzed using SPSS package (Version 17.0). Differences were considered significant at $P<0.05$ [13].

IV. Results and Discussion

The antioxidant activity of raw and cooked pool barb are shown in Figs. 1 and 2. The IC50 value of reference ascorbic acid was 46.66µg/ml. The antioxidant activity was highest in the curried (0.59µg/ml) and lowest in the steamed (16.09µg/ml) samples. Many proteins have been shown to have antioxidative activity against peroxidation of lipids or fatty acids. Kawashima [14] investigated the effects of many synthetic peptides on lipid oxidation and found that some peptides having branched-chain amino acids (valine, leucine and Isoleucine) showed antioxidative activity.

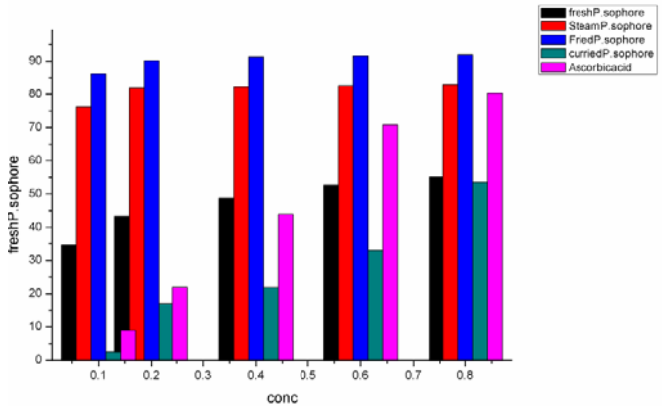

Fig. 1 Antioxidant properties of raw and cooked Pool barb

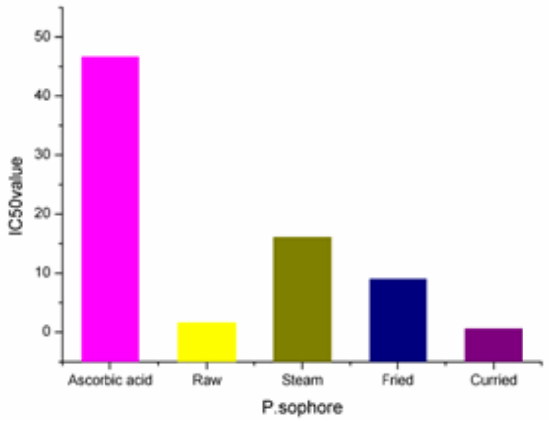

Fig. 2 IC_{50} value of raw and cooked Pool barb

The peptides containing basic amino acids are electron acceptors that take electrons from radicals formed during the oxidation of unsaturated fatty acids. Shailaja [15] reported the seer fish protein has exhibits the antioxidant activity. The total amino acids of raw and cooked pool barb were shown in Table I. The curried samples were found highest (288.43±0.12) and lowest in fried (36.79±0.02) samples. Amino acids are building blocks for the synthesis of proteins, including antioxidant enzymes. Some amino acids (e.g., arginine, glycine and histidine), small peptides and nitrogenous metabolites directly scavenge oxygen free radicals [16]. Amino acids are also suggested to have antioxidant properties as reaction products with carbonyls from oxidizing lipids. Various studies have shown results that suggest that reactions between oxidized lipids and amino acids produce many non-enzyme browning reaction compounds, which exert antioxidative properties [17].

Proximate composition of raw, steamed, fried and curried of *Puntius sophore* are shown in Fig. 3. Protein content was 20.50±0.08 in raw, 19.76±0.10 in fried was slightly decreased from raw and increased from curried (18.66±0.13). The highest protein was recorded in steamed samples (21.56±0.10). Moisture content in raw, steamed and curried 76.35±0.09%, 71.28±0.13 and 57.46±0.24 were obtained respectively. The minimum moisture was observed in fried fish (46.27±0.14). Lipid content was found highest in fried 36.35±0.14, curried 21.76±0.10 and lowest in steamed 0.91±0.01.

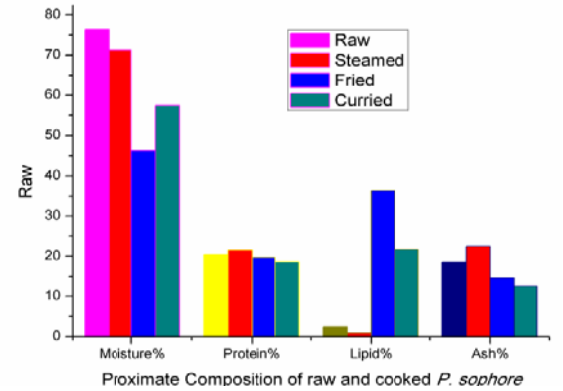

Fig. 3 Proximate Composition of raw and cooked Pool barb

TABLE I
TOTAL FREE AMINO ACIDS OF RAW AND COOKED POOL BARB (*PUNTIUS SOPHORE*) VALUES ARE SHOWN AS MEAN±STANDARD ERROR OF TRIPLICATES

Puntius sophore	Total free amino acids
Raw	196.53±0.14c mg/g
Steamed	41.04±0.01b mg/g
Fried	36.79±0.02a mg/g
Curried	288.43±0.12d mg/g

The lipid content of fried fish had higher fat than the raw fish. Ash content was highest in steamed 22.53±0.07 and lowest in curried 12.57±0.11. The ash content of fried fish was (14.68±0.09) found decreased from raw and increased from curried. The proximate composition of fish was affected by the cooking methods. Fried fish had a higher level of fat ion than raw or other cooked fish. The increase in fat content of the fried fish fillets is also related to oil absorption during the cooking process. Fat increase can be due to the oil penetration into the food after water is partially lost by evaporation [18]. Similar results have been reported for African Catfish fried in sunflower oil [19]. The lower fat content in the curried *P. sophore* is mainly due to absorption of water used in the curry. The absorption of water is evident when we compare the fried *Puntius sophore* similar reports have been found from Arabian Gulf fish and shrimps. The decrease in the moisture content makes the protein and fat content increase significantly in cooked fish [20]. During cooking of fish products, chemical and physical reactions take place that improve or impair their nutritive value. Cooking induces water loss in the food, but in turn increases its lipid content in most cases and only some fat is loss in the case of the oiliest fish. Moreover, this effect is also dependent on the type of cooking [21]. After cooking, an important water loss was found by Weber [22] in Silver catfish and [19] in African catfish. Water loss can be explained in terms of denaturation of sarcoplasmic and myofibrillar proteins and disruption of the muscle structure, this leading to a decreasing water holding capacity of the protein fraction. All proteins are first denatured then coagulated by heat. The temperature of coagulation increases

with the addition of other ingredients. Cooking results in softening of proteins in foods as water is bound in the process of coagulation [23]. The amount of loss is probably related to the composition of muscle, denaturation of proteins by the ionic strength of the extra cellular fluid and oxidation of lipids, which decreases the solubility of proteins. The reactions of water/oil with food items particularly at high temperature as obtained during frying have been shown to affect some nutrients in the food item as well as causing alteration of the structure of the oil and denaturing of the food nutrients [24] hence the significant difference recorded in moisture content after the different processing methods. The ash content was highest in raw, this might be due to its higher bony consistency and high scaly nature. Such fish offer minerals in their edible forms more abundantly than large-sized fish do [25].

The mineral elements content of raw and cooked Pool barb were shown in Fig. 4. The Fe, Mn, Mg, Na content was highest significantly in fried and curried. The Cu, Zn, Ca, Co, K was found highest in steamed. Ni and Cr was highest in raw. The Fe content of raw fish was 152mg/100g. The change in Fe content after cooking for methods was found to be significant ($p<0.05$).The Na content of raw and fried fish was 82.24±0.10mg/100g and 101.21±0.07mg/100g. A similar finding was reported by [19] indicating significantly increased Na content in fried and grilling African catfish. The Ca content of raw fish was 902.mg/100g. However, this value is higher than that reported by other authors [26], [27]. The changes in Ca content of all samples were significant ($p<0.05$). The Cu content of raw was 4.0±0.02mg/100g. The Zn content of raw was mg/100g. The Co content of fish was mg/100g.The K content of raw was mg/100g. The mineral content of fish makes fish unavoidable in the diet as it is a source of different minerals that contribute greatly to good health. The mineral elements were recorded variation in their concentration of fish sample [28]. Windom [29] attributed such variations to the chemical forms of the elements and their concentration in the environment. The lack of Zn could result in a weakness immunity system, depression, impaired vision, taste and smell disorder impotence. However, big quantities of these elements are harmful to the human body and can result in a cellular metabolism disorders. Cobalt is known for its component of the vitamin B-complex. Numerous aspects of cellular metabolism are zinc dependent. Zn is essential for human health and well-being. It has a structural and functional role in a large number of macromolecules and is required for over 300 enzyme reactions. Zinc ions participate in all aspects of intermediary metabolism, transmission, and regulation of the expression of genetic information, storage, synthesis, and action of peptide hormones and structural maintenance of chromatin and bio membranes. Zn is thus needed for growth and development, protein and DNA synthesis, neuro-sensory functions, cell mediated immunity, thyroid and bone metabolism. Long term marginal intakes of Zn coupled with decreased absorptive efficiency could severely compromise.

Indeed, a moderate deficiency of Zn is often observed in elderly subjects [30], even in industrialized countries. About 90% of the total brain Zn is tightly bound to metalloproteinase. Zn in the adult brain is located in the cerebral cortex [31], the thinking part of the brain.

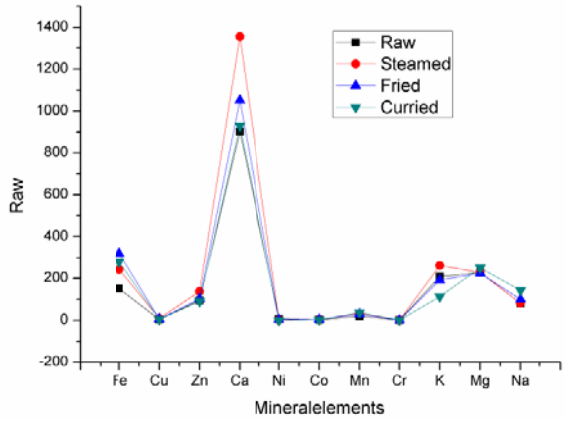

Fig. 4 Mineral elements contents of Pool barb (*P. sophore*)

V. Conclusion

The results suggest that the methanol extracts of small fishes displayed high antioxidant activity. The fish is a good source of essential mineral contents and nutritive value.

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