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Effect of Cooking on the Polyunsaturated Fatty Acid and Antioxidant Properties of Small Indigenous Fish Species of the Eastern Himalayas

Wahengbam Sarjubala Devi*, Ch. Sarojnalini**
*(Department of Life Sciences Manipur University, Canchipur, Manipur-795003)
** (Department of Life Sciences Manipur University, Canchipur, Manipur-795003)

ABSTRACT
The effect of cooking method on the polyunsaturated fatty acid and antioxidant properties of small indigenous freshwater fish species, *Amblypharyngodon mola* and *Puntius sophore* of the Eastern Himalayas were determined. In the raw and fried samples, docosahexaenoic acid was significantly higher (2.907 and 1.167mg/100g) in *Amblypharyngodon mola* and lowest (0.749 and 0.291mg/100g) were recorded in *Puntius sophore*. The eicosapentaenoic acid of raw, fried and curried samples of *Amblypharyngodon mola* were recorded higher. In DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging assay of IC_{50} value of the raw fish extract were 2.9µg/ml and 1.66µg/ml respectively. The highest antioxidant activity was found in fish curry of *Amblypharyngodon mola* (0.11µg/ml). It shows that the Maillard reaction product forms the melanoidin during cooking, increases the antioxidant property of the fish curry and also improved the taste.

Keywords – Small indigenous fishes, polyunsaturated fatty acids, antioxidant activity, cooking methods, Eastern Himalayas

I. INTRODUCTION

Amblypharyngodon mola commonly known as Mola Carplet and *Puntius sophore* (pool barb) are freshwater small fishes; a natural inhabitant of ponds, streams, ditches, beels, reservoirs and inundated fields. These species are distributed in India, Bangladesh, Pakistan and Myanmar [1]. These fishes belong to the family Cyprinidae and are one of the small indigenous freshwater fish species (SIFFS). Small Indigenous freshwater Fish species are defined as fishes which grow to the size of 25-30cm in mature or adult stage of their life cycle [2]. Traditionally, SIFFS are cooked as a whole with or without vegetables to form a curry savoured by the local people of the Eastern Himalayan region. The Eastern Himalayas are rich biodiversity region with many of the small indigenous fish being very high nutritional value. Cooking is the art of preparing food for consumption with the use of heat. There is a chemical processes central to cooking include the Maillard reaction. The Maillard reaction occurs when the denatured proteins on the surface of the recombine with the reducing sugars present. The combination creates the "meaty" flavour and changes the colour. For this reason, it is also called the browning reaction. Cooking methods are important parameters for chemical composition and nutritive value of fish muscle. An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to oxidizing agents. Oxidation reactions can produce free radicals [3]. It has been established that antioxidants found in large quantities in the crude extracts of fruits, herbs, vegetables, cereals and other plant materials act as reducing agent and thereby improve the quality and nutritional value of the food. The importance of the antioxidant constituents of plant materials has also been established in the maintenance of health by acting against stress related diseases such as infections, diabetes, cancer and coronary heart disease [4]. Fatty acids are the building blocks of the fat in our bodies and in the food we eat. Essential fatty acids include the "linolenic acid", the omega-3 family, and the "linolenic acid", the omega-6 family. Linolenic acid is a major component of the communicating membranes of the brain and is active in the eye retina. It is essential for growth and development. Nutritional components of fish have functional effects on human health. Fish is known to contain certain polyunsaturated fatty acids that can regulate prostaglandin synthesis and hence induce wound healing w-3 and w-6 PUFA have been shown to have positive effects on cardiovascular diseases and cancers [5]. The effects of different cooking methods and nutritive values of different fish species have been previously studied [6-8].

II. Materials and Methods

2.1. Sample collection

Fresh *Amblypharyngodon mola* and *Puntius sophore* were collected from Imphal and Moreh

market of Manipur, Uzzan market of Guwahati, Assam, Lamphalong, Myanmar and other different markets of the Eastern Himalayas. The vegetables like *Alocasia indica*, tomato and pea were purchased from Imphal market and brought to the Life Sciences Department, Manipur University.

2.2. Sample preparation and cooking

1kg of fish was divided into four equal lots, each lot equivalent to 250g. The first lot was uncooked while the remaining lot was cooked in the following methods i.e. frying, steaming, currying and before currying the fish was fried. The frying of fish was carried out in a frying pan of 2 litre capacity at temperature 180^0C for 4 minutes. Soyabean oil was used for pan-frying. Fried fish was cooked with chopped vegetables for 35min. Steam cooking was done in pressure cooker. After cooking process, the fishes were taken out and used for various analyses. The fish in each lot were homogenized using a mortar and pestle and analyzed to determine Antioxidant property and polyunsaturated fatty acids. All assays were conducted on triplicate samples of the homogenates.

2.3.Sample preparation for *in vitro* experiments of antioxidant properties

Fresh and cooked fish extracts were prepared in 90% aqueous methanol solution. Fresh *Amblypharyngodon mola* and *Puntius sophore* was washed with tap water and blotted dry. The vegetable which was added in fish curry preparation was removed, and 1g of raw and cooked fish was made into a paste using mortar and pestle. The paste was made a final concentration of 100mg/ml and homogenized separately for each of the samples. The homogenates were collected and centrifuged at 3,000 g for 10 min to get a clear supernatant. Finally, the clear supernatant was decanted and filtered through Whatman No.1 filter paper and stored at 4 °C for subsequent use. All assays were conducted on triplicate samples of the homogenates.

2.4. Determination of Antioxidant Properties and DPPH Scavenging Activity

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

2.5. Sample preparation for *in vitro* experiments of polyunsaturated fatty acids content

2.5.1. Extraction of sample

The raw and cooked fish tissue is macerated with Bloor's mixture (ethanol-ether 3:1) in a mortar and pestle and transferred into a glass stopper measuring cylinder and left overnight. It is then made up to the mark and filtered into a polyethene bottle through Whatman No.1 filter paper.

2.5.2. Polyunsaturated fatty acids content

Polyunsaturated fatty acids content was done following the method of AOAC [10]. 2ml of the sample solution is transferred to a reaction tube and the solvent is evaporated. Absolute ethanol and KOH glycol reagent are added and mixed thoroughly. The air in the tube is removed by heating 180^0C. A tube with only reagent and ethanol, which serves as a blank, is run with each set of samples.

2.6. Statistical Analysis

The data were analysed 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 analysed using SPSS package (Version 17.0). Differences were considered significant at $p<0.05$ [11].

III. Results and Discussion

The antioxidant activities of raw and processed *Puntius sophore* and *Amblypharyngodon mola* are shown in Table 1 and 2. The inhibition at 50% (IC_{50}) value of reference ascorbic acid was 46.66µg/ml. The co-relation coefficient value of raw *Puntius sophore and Amblypharyngodon mola* were $r^2 = 0.8878$, and $r^2 = 0.8252$. The highest antioxidant activity was found in raw *Amblypharyngodon mola* due to lesser IC_{50} value of the fish. The higher the IC_{50} value lesser the antioxidant properties. The *Puntius sophore* has also had the antioxidant activity when compared to *Amblypharyngodon mola*. This result indicates that

the raw fish extract have antioxidative properties. DPPH is a chromogen-radical- containing compound that can directly react with antioxidants. When the DPPH radical is scavenged by antioxidants through the donation of hydrogen to form a stable DPPH-H molecule, the colour is changed from purple to yellow [12]. Stable radical DPPH has been widely used for the determination of primary antioxidant activity, that is, the free radical scavenging activities of pure antioxidant compounds, plant and fruit extracts, and food materials [13].

Table 1: Antioxidant properties of raw and processed *Puntius sophore*

Puntius sophore	IC_{50} (µg/ml)
Ascorbic Acid	46.66
Raw	16.09
Steam	8.99
Fried	0.59
Curried	1.66

Table 2: Antioxidant properties of raw and processed *Amblypharyngodon mola*

Amblypharyngodon mola	IC_{50} (µg/ml)
Ascorbic Acid	46.66
Raw	2.99
Steam	25.10
Fried	16.39
Curried	0.11

The co-relation coefficient (r^2) value of steamed *Puntius sophore* and *Amblypharyngodon mola* were $r^2 = 0.5022$ and $r^2 = 0.6807$. The IC_{50} values of steamed *Puntius sophore and Amblypharyngodon mola* were 8.99µg/ml and 25.10µg/ml. The results suggest that the all steam fishes have antioxidant activities. The steamed *Puntius sophore* has the antioxidant properties and when compared to steam *Amblypharyngodon mola* it was found higher antioxidant properties. This result indicates that the steamed cooked fish extracts have antioxidative properties. The co-relation coefficient value of fried *Puntius sophore and Amblypharyngodon mola* were $r^2 = 0.6665$ and $r^2 = 0.242$. The IC_{50} values of fried *Puntius sophore and Amblypharyngodon mola* were 0.59µg/ml and 16.39µg/ml. The highest antioxidant activity was found in *Puntius sophore* due to less IC_{50} value. The results suggest that the fried fish extracts have antioxidant potential to scavenge the free radicals.The co-relation coefficient value of curried *Puntius sophore and Amblypharyngodon mola* were $r^2 = 0.9507$ and $r^2 = 0.7842$. The IC_{50} values of *Puntius sophore and Amblypharyngodon mola* were 1.66µg/ml and 0.11µg/ml. The highest antioxidant activity was found in curried *Amblyphayngodon mola*. The results are in agreement with those obtained from the antioxidant activity determined by the DPPH radical scavenging assay. Compounds responsible for reducing activity are formed during the thermolysis of Amadori products in the primary phase of Maillard reaction [14] or they could be formed by heterocyclic compounds of Maillard reaction [15]. The results revealed that the Maillard reacted products (MRPs) could function as electron donors. The hydroxyl groups of MRPs play an important role in reducing activity [16]. Additionally, the intermediate reductone compounds of MRPs were reported to break the radical chain by donation of hydrogen atom [17-18]. Table 3 and 4 shows the polyunsaturated fatty acid contents raw and processed fishes of *Puntius sophore* and *Amblypharyngodon mola*.

The polyunsaturated fatty acid (PUFA) consists of Docosahexaenoic acid (DHA), Eicosapentaenoic acid (EPA), Arachidonic acid (AA), Lenolenic acid (ALA) and Lenoleic acid (LA). In raw fish, DHA was significantly highest (2.907mg/100g) in *Amblypharyngodon mola* and lowest was (0.749mg/100g) in *Puntius sophore.* The EPA, AA, lenolenic and linoleic acid are the essential fatty acids which mean that our bodies are not able to synthesize the compounds they are provided with the diet. Among the PUFA, the most dominant was DHA in all raw fish samples. The present study shows that a total PUFA level varies among the species. Fish oil is considered as liquid oil, but, in fact contains triglycerides of intermediate melting point for the oils to be partially solid at 20^0C. Fish oils are unique in the variety of fatty acids of which they composed and their degree of un-saturation [19]. Polyunsaturated fatty acids composition may vary among species of fish, even among fresh water and marine fish [20]. The Omega-3 and Omega-6 polyunsaturated fatty acids (PUFA) have been shown to have positive effects on Cardiovascular and Cancers [21].

The contents of DHA, EPA and AA of steamed *Amblypharyngodon mola* and *Puntius sophore* were ranged from 1.3 to 2.907mg/100g, 0.863 to 1.757mg/100g, 0.08 to 0.097 mg/100g. In steamed fishes their levels of DHA were decreased significantly ($p<0.05$) from raw in *Amblypharyngodon mola* and increased significantly ($p<0.05$) in raw *Puntius sophore* (2.907mg/100g). The level of EPA was increased significantly ($p<0.05$) in all fish samples.

Table 3: Polyunsaturated fatty acid (PUFA) of raw and cooked *Puntius sophore*

PUFA	Raw	Steam	Fried	Curried
DHA	0.75 ±0.00[b]	2.92 ±0.00[d]	0.28 ±0.00[a]	1.93 ±0.00[c]
EPA	0.04 ±0.00[a]	1.73 ±0.00[d]	0.12 ±0.00[b]	0.15 ±0.00[c]
AA	0.06 ±0.00[a]	1.90 ±0.00[d]	0.21 ±0.00[b]	0.31 ±0.00[c]
Lenolenic Acid	0.91 ±0.00[a]	1.89 ±0.01[b]	3.02 ±0.00[c]	3.06 ±0.00[d]
Lenoleic Acid	2.19 ±0.00[d]	1.36 ±0.00a	1.90 ±0.00[c]	1.43 ±0.00b

Values are shown as mean±standard error of triplicates.
Values within the same row have different superscripts are significantly differences *(P<0.05)*

Table 4: Polyunsaturated fatty acid (PUFA) of raw and processed *Amblypharyngodon mola*

PUFA	Raw	Steam	Fried	Curried
DHA	2.91 ±0.00[d]	1.37 ±0.00[b]	1.15 ±0.00[a]	1.72 ±0.00[c]
EPA	0.12 ±0.00[a]	0.86 ±0.00[d]	0.50 ±0.00[b]	0.73 ±0.00[c]
AA	0.13 ±0.00[b]	0.09 ±0.00[a]	2.03 ±0.00[d]	1.83 ±0.00[c]
Lenolenic Acid	1.40 ±0.00[b]	1.08 ±0.00[a]	2.31 ±0.00[c]	2.86 ±0.00[d]
Lenoleic Acid	2.30 ±0.00[d]	2.03 ±0.00[c]	1.35 ±0.00[b]	1.01 ±0.00[a]

Values are shown as mean±standard error of triplicates.

Values within the same row have different superscripts are significantly differences *(P<0.05)*

The levels of AA were decreased in steamed *Amblypharyngodon mola* (0.86mg/100g). The level of AA was increased significantly (p<0.05) in steamed*Puntius sophore* (1.73mg/100g) as compared to the raw. The level of lenolenic acid was increased significantly (p<0.05) in steamed *Puntius sophore*(1.89mg/100g) and decreased significantly (p<0.05) in steamed *Amblypharyngodon mola* (1.08mg/100g). The level of linoleic acid was decreased significantly (p<0.05) in all raw and cooked samples. In fried samples the level of DHA were decreased significantly (p<0.05) in all samples. The level of EPA, AA and lenolenic acid were increased significantly (p<0.05) in all the fish samples. The level of linoleic acid was decreased significantly (p<0.05) in fried *Amblypharyngodon mola*(1.35mg/100g) and increased significantly (p<0.05) in fried *Puntius sophore* (1.9mg/100g).

In curried samples, the level of DHA was increased significantly (p<0.05) in curried *Puntius sophore*(1.93mg/100g) and decreased significantly (p<0.05) in curried *Amblypharyngodon mola*(1.72mg/100g). The levels of EPA, AA and lenolenic acid were increased significantly (p<0.05) in all the fish samples. The level of linoleic acid was decreased significantly (p<0.05) in curried *Amblypharyngodon mola*(1.01mg/100g) and increased significantly (p<0.05) in curried *Puntius sophore*(1.43mg/100g). The changes in amounts of EPA and DHA observed in the results were caused by changes in amounts of water and fat rather than a consequence of applying heat during cooking. The changes in lipid extractability had also some influence on the amount of measured EPA and DHA, what in turn directly affected percentages and composition of fatty acids. Alkanes/alkenes, alcohols, aldehydes and ketones may be produced by thermal oxidation and degradation of polyunsaturated fatty acid [22-23]. Results illustrated that there were obvious qualitative and quantitative differences between raw and cooked flavour were affected by methods of cooking before serving. Most of the flavour compounds represented is well known in lipid oxidation products. There were some compounds that generated via lipoxygenase, amino acid degradation and Maillard reaction. Aroma generated during heat process can be affected by lipid content and fatty acid composition of food. High proportions of unsaturated fatty acids in fish can give more unsaturated volatile aldehydes. Guizani *et al.* [24], reported that in smoked Tuna polyunsaturated fatty acids (PUFAs) significantly decreased after smoking. Kaya *et al.* [25], also reported the similar effect of hot smoking in the fatty acids of sturgeon. The recommended daily intake of EPA and DHA is 1g/day [26-27], noted that n-3 PUFA, principally DHA, has a role in maintaining the structure and functional integrity of fish cells. In addition, DHA has a specific and important role in neural (brain and eyes) cell membranes. Moreover, DHA is considered a desirable property in fish for human nutrition and health. Fish lipids, due to high amounts of unsaturated fatty acids and low amounts of natural antioxidants, auto-oxidises at a much more rapid rate than other kinds of lipids [28-29]

IV. Conclusion

From the above investigation it was found that the extracts of fish species (*Puntius sophore and Amblypharyngodon mola*) have noticeable antioxidant activities. It has the ability to scavenge the free radical. The small indigenous fish species had good amount of PUFA content especially omega-3 fatty acids that are likely to lower the risk of heart diseases in adults and support good health and neuro-development in infants and young. Small fishes are a valuable and easily available source of food rich in omega-3 fatty acids and natural antioxidant

properties. Hence, the consumption of small indigenous fishes should be encouraged.

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