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Effect of Maillard Reaction on the Antioxidant properties and Amino acid content of *Amblypharyngodon mola* of the Eastern Himalayas

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Abstract

The effect of Maillard Reaction on the antioxidant activity and total amino acid contents of the processed *Amblypharyngodon mola* of the Eastern Himalayas was determined. The *Amblypharyngodon mola* (family-Cyprinidae) is locally known as Mukanga in Manipur and mowa in Assam, mowa or mowka in west Bengal. The small fishes are consumed as a whole; they are often cooked in fish alone and also with vegetables. In the present study the fish was cooked in different forms i.e., steaming, frying and fish curry. The highest antioxidant activity was found in fish curry as compared to fresh, steamed and fish fried. The chemical reaction occurred during cooking process is the Maillard reaction i.e., the condensation reaction between the amino acids and carbonyl carbon of the reducing sugar present inside the fish body forms the melanoidin (Heterocyclic nitrogenous compound) compounds. The highest total amino acid was found in steamed as compared to other processed samples. The results suggested that the Maillard reaction product increases the amino acids content and antioxidant property of the steamed and fish curry and also improved the taste of the product. Thus consumption of the processed small fishes should be encouraged.

Keywords- Antioxidant activity; Amblypharyngodon mola; Small indigenous freshwater fish; Eastern Himalayas

Introduction

Amblypharyngodon mola, commonly known as Mola Carplet is a freshwater small indigenous fish species. They are natural inhabitant of ponds, streams, ditches, beels

and reservoirs. The species is distributed in India, Bangladesh, Pakistan and Myanmar (Talwar and jhingran,1991). In West Bengal, Manipur and Assam it is locally known as mowa or mowka, Mukanga and Mowa respectively. It is a popular food fish in West Bengal due to its good taste as well as high nutrient value with presence of good amount of vitamin A, protein and mineral contents (Zafri and Ahmed,1981;Saha *et al.*, 2009). The small fishes are consumed as a whole; they are often cooked in fish alone and also with mix vegetables. Antioxidants are free radical scavengers which protects the human body from the pathological effects of free radicals. These chemical compounds are found in various processed foods or medicines as preservatives (Oyaizu, 1986).

Cooking or cookery is the art of preparing food for consumption with the use of heat. Cooking techniques and ingredients vary widely across the world, reflecting unique environmental, economic, and cultural traditions and trends. The application of scientific knowledge to cooking and gastronomy has become known as molecular gastronomy. This is a sub discipline of food science. There is a chemical processes central to cooking include the Maillard reaction - Maillard reaction is a non-enzymatic interaction between reducing sugar and amino acid, peptide or protein, resulting in a variety of by-products, intermediates and brown products (melanoidins), which contribute markedly to the aroma, taste and colour, as well as to the antioxidant potential of stored and processed foods (Manzocco et al., 2001). It is one of the major reactions taking place during thermal processing, cooking and storage of foods. In 1912 Louis Camille Maillard described a browning reaction between reducing sugars and amino groups. The chemistry of the Maillard reaction is known as a complex series of reactions leading to the formation of variety of products, including flavours, aromas and colours considered important in food Science today. Most foods are only when they are cooked. Except for some fruits and vegetables, most of the food we eat is cooked. For centuries we have nurtured this art of cooking. One of the foremost reasons for cooking is that it improves taste, flavour, colour and also its nutritive value.

In Manipur there are some reports on fresh and processed fishes (Sarojnalini and Vishwanath, 1988; Sarjubala and Sarojnalini, 2012). However, there is no report so far on effects of cooking/Maillard reaction on the Antioxidant properties and total amino acid content of processed small fishes used in our daily life. Thus, this study was carried out to determine the effect of cooking reaction on the Antioxidant properties and total free amino acid content of processed *Amblypharyngodon mola* of the Eastern Himalayas.

2. Materials And Method

2.1 Sample collection

Fresh *Amblypharyngodon mola* (Figure 1) was collected from Manipur, Assam and Myanmar of the Eastern Himalayas. The vegetables like *Alocasia indica*, tomato and pea were purchased and brought to the Life Sciences Department, Manipur University.

2.2 Sample preparation and cooking

Fresh fishes and vegetables were washed with tape water. 1kg of fish was divided into four equal lots, each lot equivalent to 250g. The first lot was uncooked it was used as control while the other three lots were cooked in the different 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 litre capacity at temperature 180^0C for 4 minutes. Soyabean oil was used for pan-frying and its temperature during the frying process was 180^0C.For curry preparation fried fish was processed with chopped vegetables for 35min. After the cooking the fishes were taken for various analyses. All fish in each lot were homogenized and used for various analyzes to determine antioxidant activity and total amino acid contents. All assays were conducted on triplicate samples of the homogenates.

2.3. DPPH radical scavenging activity

The antioxidant activities have been substantially investigated using 1,1-Diphenyl-2-picrylhydrazyl (DPPH) scavenging assay following the method of Cuendet *et al*., 1997. DPPH radicals are stable radicals and in the presence of molecules capable of donating Hydrogen atoms, its radical character is neutralized. DPPH scavenging assay is widely used for assessing the ability of radical scavenging activity and it is measured in terms of Inhibition 50% (IC_{50}) values. Due to the presence of odd electron, DPPH shows a strong absorption band in 517 nm in the visible spectrum. As this electron becomes paired off in the presence of a free radical scavenger, absorption band at 517 nm vanishes, and the resulting decolouration is stoichiometric with respect to the number of electrons taken up. 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 radical scavenging assay, expressed as percentage of inhibition was calculated by using the following equation:

$$\text{DPPH decolouration (\%)} = (1\text{-ODs} / \text{ODc}) \times 100$$

The degree of decolouration indicates the free radical scavenging efficiency of the substances. Where, ODs= Optical density of sample and ODc = Optical density of control.

2.4. Total amino acid content

The total free amino acids were determined according to the Moore and Stein, 1948. 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.

2.5. 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 $P<0.05$ (Sokal and Rohlf, 1974).

Table 1. Antioxidant activity and total free amino acid content of raw and processed Amblypharyngodon mola.

Amblypharyngodon mola	Total free amino acid(mg/g)	Antioxidant activity (µg/ml)
Raw	251.03±0.00	2.9±0.00
Steamed	454.03±0.00	25.10±0.00
Fried	143.2±0.00	16.39±0.00
Curried	90.69±0.00	0.11±0.00

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

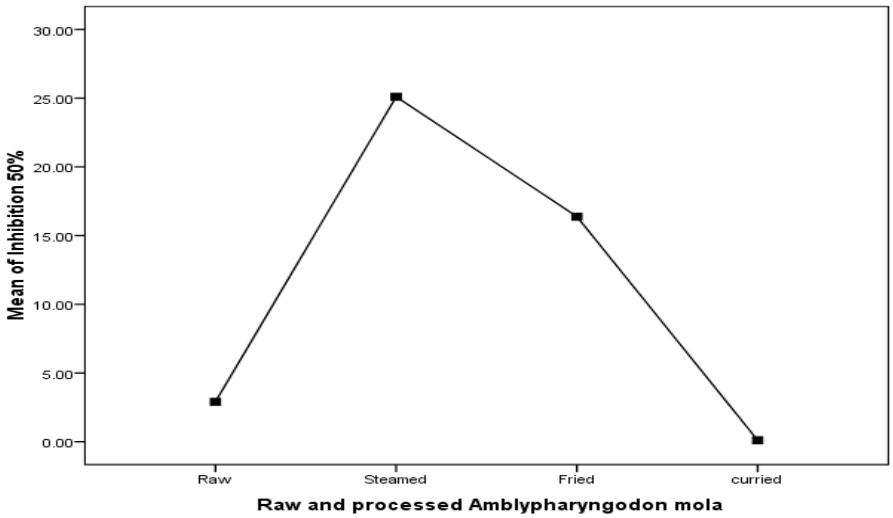

Figure 1. Antioxidant activity of Raw and processed *Amblypharyngodon mola.*

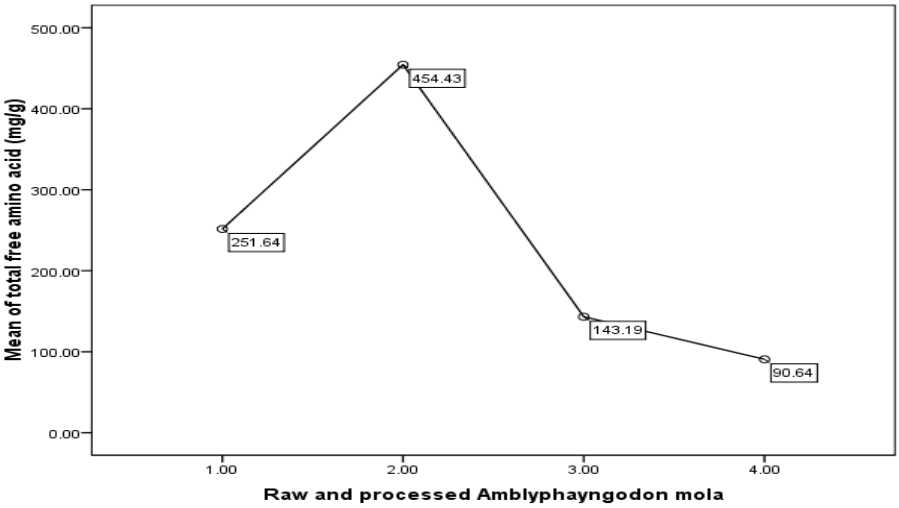

Figure 2. Total free amino acid content of raw and processed *A.mola.*

Results And Discussion

The antioxidant activity and IC_{50} value (Inhibition 50%) of processed Mola carplet (*Amblypharyngodon mola*) are shown in table 1 and fig. 1. The IC_{50} value of reference ascorbic acid was 46.66µg/ml and lesser the IC_{50} value higher the antioxidant properties. The antioxidant activity was highest in the curried (0.11µg/ml) and lowest in the steamed (25.1µg/ml) samples. The antioxidant activity was also found in raw (2.9µg/ml) and fried (16.391µg/ml) samples. This activity was increased by increasing the concentration of the sample extract. DPPH antioxidant assay is based on the ability of 1,1-diphenyl-2-picryl-hydrazyl (DPPH), a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm and also for a visible deep purple colour. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance. Many proteins have been shown to have antioxidative activity against peroxidation of lipids or fatty acids. Kawashima *et al*., 1979 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.

The total free amino acids of raw and processed *A.mola* were shown in table 1 and fig. 2.The steamed samples was found highest (454.43±0.00) and lowest in fish curry (90.64±0.00) samples. The total free amino acids of raw and fried samples are (251.03±0.00) and (143.2±0.00). 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 (Wu and Meininger, 2002). 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 (Alaiz et al., 1995, Alzaiz, et al., 1996 and Ishtiaque, et al., 1996). The Maillard reaction, produced from an amino acid-sugar model system, has been known to be associated with the formation of compounds with antioxidant properties. The Maillard reaction is initiated by a condensation reaction between the carbonyl group of the aldose and the free amino group of an amino acid to give an n-substituted aldosylamine. This is the result of a nucleophilic attack group by the amine (NH_2) group of the amino acid on the electrophilic carbonyl groups of reducing sugar. The condensation product rapidly loses water as a product and is converted into a Schiff base (C=N). The Schiff bases then cyclize into the aldosylamine. The amadori rearrangement follows to form a ketosamine. The Amadori rearrangement is considered to be the key step in the formation of major intermediates for the browning reaction. The Strecker degradation of amino acids involves their oxidative degradation by carbonyl compounds, which arise from the degradation of ketosamines. In this degradation reaction, amino acids first react to form Schiff bases and undergo acid catalysed decarboxylation. The new Schiff base is easily hydrolysed to form an amine and aldehyde. Strecker degradation is characterized by the

production of CO_2 and the net result is a transamination reaction which might be important for the incorporation of nitrogen into melanoidins. The formation of melanoidins is the result of the polymerization of highly reactive intermediates that are formed during the advanced Maillard reaction (Mauron, 1981).

3. Conclusions

In conclusion, considering the results obtained from antioxidant activity and total free amino acid content analysis, the methanol extract of the processed *A. mola* contains promising natural antioxidative compounds and that might be responsible for the therapeutic activity of the fish extract in the treatment of various diseases. Further research to isolate and characterize the organic constituents may enhance the scientific basis of its uses in traditional medicine.

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