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Excessive consumption of certain sea fishes and mushrooms may pose significant health risks

M. Seth, P. Mondal, D. Ghosh and S. K. Mukhopadhyay*

Department of Microbiology, The University of Burdwan, Burdwan, Purba Bardhaman, West Bengal, India, 713104

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ABSTRACT

Trimethylamine N-oxide (TMAO) is a diet-dependent molecule that causes atherosclerosis in human bodies. Elevated TMAO levels in the blood of type 2 diabetes and chronic renal disease patients have also been documented. This study examines trimethylamine (TMA) and TMAO levels in several edible mushrooms and marine fishes. According to the quantitative study, TMA and TMAO levels are high in the fish and mushroom samples. Although the examined marine fishes and mushrooms are considered nutritious and healthy, our data indicate that significant TMA/TMAO in those healthy foods may pose major health risks if ingested in excess.

Keywords: Trimethylamine N-oxide; Trimethylamine; Atherosclerosis; Sea fishes; Mushrooms

Introduction

Occurrences of cardiovascular diseases (CVD), type 2 diabetes, and chronic kidney disease are increasing day by day, taking millions of lives every year. The gut microbiota, also known as our second brain, contributes to those ailments by forming TMAO in our body (Velasquez *et al.* 2016). TMAO acts as an osmoregulator in marine organisms, protecting their tissues from high salinity of sea, pH, effects of hydrostatic pressures, and high urea concentrations. TMAO, considered beneficial for marine fish, has become a primary human health concern due to its involvement in atherosclerosis (Velasquez *et al.* 2016). A high protein diet forms trimethylamine (TMA) by microbial TMA lyase enzymes of the human gut, which is further converted to TMAO by hepatic flavin-containing monooxygenase (FMO3) enzyme. From the liver TMAO enters the bloodstream, alters cholesterol metabolism, and blocks the artery by activating and depositing foam cells, leading to endothelial dysfunction, forming unstable plaques in the arterial wall, which results in atherosclerosis (Zhu *et al.* 2020). High TMA levels cause Trimethylaminuria (TMAU) or 'fishy odor syndrome' resulting in rotten fish odor in sweat, breath, and urine in patients who cannot metabolize TMA to TMAO due to mutation or lack of FMO3 gene (Zhu *et al.* 2020). Prolonged consumption of TMA-forming food in large quantities has increased blood pressure levels and kidney damage by up-regulating the markers of KIM-1 and proteinuria in rats (Maksymiuk *et al.* 2022). TMA and TMAO generated in the human system are filtered and excreted out by kidneys, however, it has been observed that the level of excretion decreases with the increase in TMAO concentration due to saturation of renal tubular secretion. So, TMAO may be necessary for developing chronic kidney disease (Tang *et al*. 2015). Experimental studies on animals have portrayed TMAO as a metabolite in impairing glucose tolerance ability, developing insulin resistance, and promoting oxidative stress in adipose tissues. A study performed in middle age and older Chinese cohorts showed that high TMAO levels were associated with a greater risk of type 2 diabetes and an increase in fasting glucose (Li *et al.* 2022).

*Corresponding author's e-mail: skmukhopadhyay@microbio.buruniv.ac.in

It is reported that ergothioneine, present in some mushrooms such as bolete, oyster etc., have antioxidant properties that contribute to the lowering of cardiometabolic morbidity and mortality, but their high TMAO content makes them questionable as healthy food (Papandreou *et al.* 2020). Present work shows TMAO content in the blood serum of CVD patients was an average value of 5µM, compared to healthy individuals with an average value of 3.5µM. Also, patients with elevated TMAO levels of 6.18µM had a higher occurrence of CVD over the years, contrary to individuals with lower TMAO levels in the blood (Jing *et al.* 2022). Reecently it has been reported that TMAO is also present in a considerable concentration in some plants as an abiotic stress regulator (Catalá *et al*. 2021).

We have spectrophotometrically quantified TMA and TMAO content of some marine fishes and mushrooms procured from local vendors to check their food quality regarding TMA/TMAO content.

Materials and methods

Collection of the samples

Four sea fishes viz *Tenualosa ilisha* (Hilsa), *Pampus argenteus* (Pomfret), *Mystus gulio* (Nuna Tengra), and *Nematalosa nasus* (Khoira) and three edible mushrooms viz. *Boletus barrowsii* (white king bolete), *Pleurotus ostreatus* (Oyster), and *Agaricus bisporus* (Button) were procured from Contai and local market, respectively, and stored in ice boxes until use.

Fish muscle and mushroom samples preparation

The sample preparation was done by dividing the fish into three sections i.e. the head, stomach, and tail, and the mushroom samples were divided into two parts, the cap, and the stem region. Extracts of different fish and mushroom parts were prepared by homogenizing l0 g of the sample in 50 mL of 10% trichloroacetic acid (TCA) solution using a borosil glass homogenizer (Wekell and Barnett, 1991). The homogenate of samples were then carefully filtered through Whatman No.1 filter paper to remove gross debris, and the clear filtrates were collected in separate tubes and used for analysis.

Quantitative TMA and TMAO assay method

TMA and TMAO quantification assays were performed according to Wekell and Barnett (1991), with slight modifications. One ml of sample extract (fish/mushroom suitably diluted as required) was taken in a screw-capped vial; in another vial 1mM of standard TMA solution was taken for TMA assay, and 1mM of standard TMAO solution was taken for TMAO assay (Wekell and Barnett, 1991). Sterile distilled water was taken in a vial as control, and diluted TCA solution was taken in another vial as blank. Both experiments were carried out in triplicate. The absorbance of the samples was measured at 410 nm in UV-VIS double beam Spectrophotometer of Shimadzu (Model – UV-1900) using the sample blank for reference. A working

Table I. The absorbance values of TMA concentrations (mM) to prepare the standard curve

TMA concentrations	Optical density at 410nm
0mM	0±0
0.25mM	0.020±0.0085
0.5mM	0.045±0.0067
0.75mM	0.060±0.0244
1mM	0.099±0.0201
1.25mM	0.132±0.0173
1.5mM	0.148±0.0157
1.75mM	0.166±0.0160
2mM	0.201±0.0136

Table II. The absorbance values of concentrations of TMAO (mM) to prepare the standard curve

TMAO concentrations	Optical density at 410nm
0mM	0±0
0.25mM	0.062±0.0159
0.5mM	0.122±0.0139
0.75mM	0.182±0.0163
1mM	0.218±0.0195
1.25mM	0.252±0.0153
1.5mM	0.305±0.0110
1.75mM	0.335±0.0044
2mM	0.423±0.0206
2.25mM	0.440±0.0235
2.5mM	0.518±0.0447
2.75mM	0.549±0.0990
3mM	0.626±0.0215
3.25mM	0.648±0.0523
3.5mM	0.662±0.0416
3.75mM	0.776±0.0371
4mM	0.796±0.0099

standard graph was prepared for known TMA concentrations of 0.25mM, 0.5mM, 0.75mM, 1mM, 1.25mM, 1.5mM, 1.75mM and 2mM (Table I) and known TMAO concentrations of 0.25mM, 0.5mM, 0.75mM, 1mM, 1.25mM, 1.5mM, 1.75mM, 2mM, 2.25mM, 2.5mM, 2.75mM, 3mM, 3.25mM, 3.5mM, 3.75mM and 4mM (Table II). The optical density values of the samples were plotted against the standard curve to quantitatively determine the content of TMA and TMAO present in various sections of fish and mushrooms.

Statistical analysis

The experiments described above were performed in triplicate. The statistical properties like mean and standard deviation of standard TMA and TMAO concentrations were prepared in Microsoft Excel and the concentration bar graphs of the samples were performed using GraphPad Prism7 software.

Results and discussion

The quantitative TMA and TMAO assay results showed the highest concentration of TMA (Fig. 1.) in Khoira tail of 2.23 mM per 200 mg among sea fishes and in cap region of Oyster mushroom of 0.31 mM per 200 mg among mushrooms. The highest concentration of TMAO among sea fishes (Fig. 2) was found in Hilsa tail at 3.69 mM per 200 mg, and among mushrooms is Oyster cap of 0.14 mM per 200 mg. This experiment shows that levels of both TMA and TMAO are quite high in both fish and

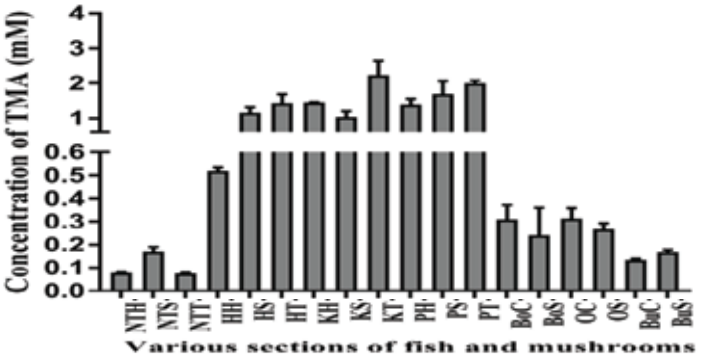

Fig. 1. This graph shows the various concentrations of TMA present in the fish and mushroom samples. [NTH, Nuna tengra head; NTS, Nuna tengra stomach; NTT, Nuna tengra tail; HH, Hilsa head; HS, Hilsa stomach; HT, Hilsa tail; KH, Khoira head; KS, Khoira stomach; KT, Khoira tail; PH, Pomfret head; PS, Pomfret stomach; PT, Pomfret tail; BoC, Bolete cap; BoS, Bolete stem; OC, Oyster cap; OS, Oyster stem; BuC, Button cap; BuS, Button stem]

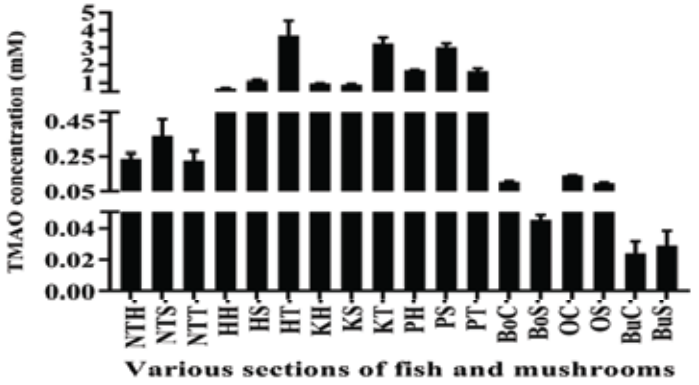

Fig. 2. This graph shows the various concentrations of TMAO present in the fish and mushroom samples

mushroom samples, and these concentrations can be detrimental if consume in high amounts leading to TMAO production in blood.

This study gives strong evidence of the presence of high content of TMAO in all the tested sea fishes and mushrooms. Our findings in mushrooms corroborate those reports. Despite TMA/TMAO, sea fishes and mushrooms are highly nutritious and popular food items. Researchers are trying various procedures to reduce TMA and TMAO in the blood by employing compounds like meldonium, 3,3-dimethyl-1-butanol, antibiotics, resveratrol (RSV), etc. (Velasquez *et al*. 2016). Unfortunately, all these compounds have many adverse effects on human health so our aim should be to reduce TMA or TMAO production by bacterial metabolism. Bacterial strains having TMA and TMAO degrading enzymes are reported, and they may be employed in the human gut to reduce the TMAO formation by degrading natural or metabolically synthesized TMA (Seth *et al*. 2023).

Conclusion

Though a considerable amount of TMA/TMAO is present in the tested sea fishes and mushrooms, considering their nutritional value, economic importance, and acceptability, it will be difficult to omit those food items as potential sources of health risk. Intermittent consumption of small quantities of tested TMA / TMAO rich food may help avoid their probable health hazard. However, these findings will pave a new way for research in this field.

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