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Detection of Food Mutagens in Processed Meat

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Abstract Among the environmental factors that may contribute to the genesis of human cancer, diet is regarded as a major determinant. It was of interest therefore to study the human exposure to heterocyclic aromatic amines found in various meat products. This study aims at a qualitative analysis of food carcinogens employing the Electrospray Ionization Mass Spectrometry. A total of three samples namely Peppered Mackerel, Chicken Salami and Bacon Grill randomly collected from the local grocery stores of New Delhi, India were analyzed for the presence of food carcinogens. Standard carcinogenic compounds namely PhIP (2-amino-1-methyl-6-phenylimidazo [4,5-b] pyridine) and MeIQx (2-amino-3,8 dimethylimidazo[4,5-f] quinoxaline) were employed as reference mutagens. Results obtained establish the presence of a potential hallmark carcinogen MeIQx whose mass corresponds to (m/z = 214.09) in Bacon Grill, concluding the detection of MeIQx. We were unable to detect either of the indicated mutagens in Peppered Mackerel and Chicken Salami respectively.

Keywords: *electrospray ionization mass spectrometry, food carcinogens, PhIP, MeIQx, cancer, processed meat*

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1. Introduction

Cancer is a growing health problem around the world particularly with the steady rise in life expectancy, increasing urbanization and the subsequent changes in environmental conditions, including lifestyle. According to a recent report by the World Health Organization, Until now more than ten million cases of cancer are reported per year worldwide. Much effort has gone into investigating the factors affecting the formation, yield and structures of such compounds [1,2]. The three major causes of human carcinogenesis are nicotine from cigarette smoking, infection and inflammation and nutrition and dietary factors [3,4,5,6,7]. The search for carcinogenic agents in foods is directed towards explaining the occurrence of cancer in different groups of humans [8,9,10]. Heterocyclic aromatic amines (HAAs) are mutagens/carcinogens formed during the cooking of muscle meat and fish as byproducts of the Maillard reaction between free amino acids, creatine/creatinine and hexoses. HAAs are considered as important factors in a lifestyle related carcinogenesis, according to the International Agency for Research on Cancer classified as 2A/B carcinogens [11,12] therefore being extensively investigated worldwide with a particular interest in finding methods to diminish their genotoxic potential. More than twenty four different type of food mutagens have been identified from cooked meat products. Studies have shown that PhIP (2-amino – 1methyl 6 phenylimidazo [4,5b] pyridine) which induces tumor of the prostate and colon in male rats and those of the colon and breast in females is the most abundant HAA formed, followed by MeIQx (2-amino 3,8-dimethylimidazo [4,5f] quinoxaline [11,12,13] which induces liver and lung tumors in mouse models. As a result, numerous studies concerning protective effects of several dietary components against the mutagenic and carcinogenic activity of HAAs have been performed. Exposure to HAAs has been implicated in the etiology of certain human cancers including colon, prostate and breast cancer. However, much remains to be investigated to determine the mechanisms by which HAAs exert their effects and to discover which HAAs have the greatest relevance to human cancer incidence [14].

Our laboratory has been engaged in standardizing methods for detection of dietary mutagens [15], We wanted to explore new methods to validate earlier findings. Therefore, in the present study, we targeted two of the most commonly occurring carcinogens in food, which were assessed employing Electrospray ionization mass spectrometry (ESI MS).

2. Materials and Methods

2.1. Chemicals

2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP, CAS No. 105650-23-5) and 2-amino-3,8 dimethylimidazo[4,5-f]quinoxaline (MeIQx, CAS No. 77500-04-0) were purchased from Toronto Research Chemicals (North York, Ontario, Canada). Amberlite XAD-2 resin was from Supelco (Bellefonte, Pennsylvania, USA). Hydrochloric acid, sodium hydroxide and acetone were purchased from Sisco Research Laboratories (Mumbai, India). All reagents were analytical grade. Methanol obtained from Merck (Mumbai, India) was of HPLC grade. The extracts were filtered before injecting

through 0.45 µm syringe filter from Axiva Sichem Biotech (New Delhi, India).

2.2. Sample Preparation

Three types of sample products namely Chicken Salami, Peppered Mackerel and Bacon Grill were purchased from local grocery stores in New Delhi, India. All the samples were stored at -20^{o}C until further processing. 50g of each product was weighed and used for processing. **Each sample was replicated twice**

2.3. Extraction of Heterocyclic Aromatic Amines from Meat Samples

Mutagens were extracted according to the method described by [16,17] with certain modifications consisting of a liquid-liquid extraction procedure at different pH followed by a solid-liquid extraction on Amberlite XAD-2 resin column. 50g of boneless meat sample was taken each time and uniformly homogenized in a double volume of distilled water in a mixer-grinder. The homogenate was acidified with 0.1M HCl to pH 2.0 and centrifuged at 6000×g for 15 minutes. The supernatant was collected and pellet resuspended in distilled water, acidified and centrifuged again. The supernatants were combined and neutralized with 1M NaOH to pH 7.0. The cloudy supernatant obtained was filtered through Whatman filter paper no1 and the clear filtrate applied to a column of Amberlite XAD-2 resin (1.5cm×10cm) at a flow rate of 2ml/min. Subsequently, 10ml of distilled water (pH 7.0) was introduced to the column and the adsorbed compounds finally eluted with 25ml of acetone followed by 25ml of methanol. Extracts were completely evaporated to dryness in a vacuum rotary evaporator and resuspended in a volume of 1ml methanol for further analysis.

2.4. Electrospray Ionization Mass Spectrometry (ESI MS)

The mass of a protein can be precisely determined by ESI MS. This is an established analytical technique [18] which is also applicable to small molecular weight compounds. Such molecules are separated according to the ratio of their mass to charge (m/z). The method used in this study was developed and validated by us. Detection of food mutagens validate the earlier reports as indicated in [19,20] Mass spectrometric graphs were recorded as intensity versus mass to charge (m/z) ratio. Technical details for the equipment used for our study were a WATERS SYNAPT G2 (HDMS), Mode employed was ESI +ve, Capillary Voltage = 3k V, Sampling Cone = - 40 k V, Extraction Cone = -120 V. Temperature of the Source = 120°C, Temperature of Desolvation = 350^{0}C. Gas flow of Cone Gas (Litres/hour) = 50 and Desolvation Gas (Litres/hour) = 600

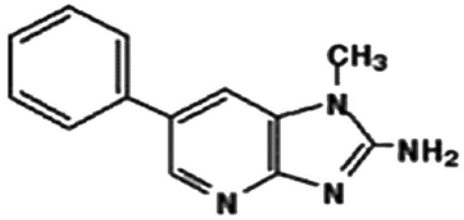
2.5. Standard Solutions for the Two Heterocyclic Aromatic Amines

A stock solution of MeIQx and PhIP (1.0 mg/ml) each was prepared by dissolving the appropriate amount in methanol. Working standards were prepared fresh by dilution of stocks in methanol so that the final concentration was (1.0mg/5ml).

3. Results

3.1. Chemical Structures of Representative Heterocyclic Aromatic Amine Compounds

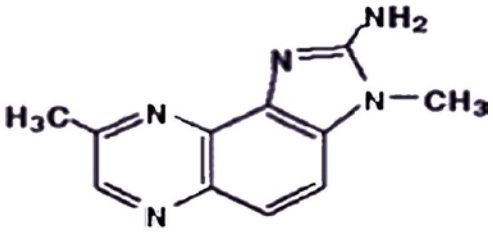
Figure 1a represents the structure of PhIP (2-amino-1-methyl-6-phenylimidazo [4,5-b] pyridine) and Figure 1b shows MeIQx (2-amino-3,8 dimethylimidazo[4,5-f] quinoxaline). Both, PhIP and MeIQx were employed as standard reference mutagenic compounds.



PhIP

2-amino-1-methyl-6phenylimidazo-[4,5-b]pyridine

Figure 1a. Chemical Structure of PhIP



MeIQx

2-amino-3,8-dimethylimidazo-[4,5-f]quinoxaline

Figure 1b. Chemical Structure of MeIQx

3.2. Nutritional Information and Ingredients

Table 1 represents the ingredients of each of the sample (Peppered Mackerel, Bacon Grill and Chicken Salami) subjected to analysis. Sample energy content, protein, carbohydrate, fat (PUFA) and other parameters are illustrated in Table 2.

Table 1. Description of samples and ingredients in selected ready to eat meat products

Sample and Ingredients		
Peppered Mackerel	**Bacon Grill**	**Chicken Salami**
Mackerel (80 %) Rapeseed Oil, Water, Sprint Vinegar, Dried Egg white, Milk proteins, Potato Fibre, Black Pepper, Flavouring, Salt, Stabilizers-Carrageenan and Locust Bean Gum.	Pork (43%), mechanically recovered pork, water, pork fat, maize starch, pork bind, salt, milk powder, stabilizer (sodium triphosphate), smoke flavor, sugar, preservative (sodium nitrite), spice extracts.	Chicken, spices, salt.

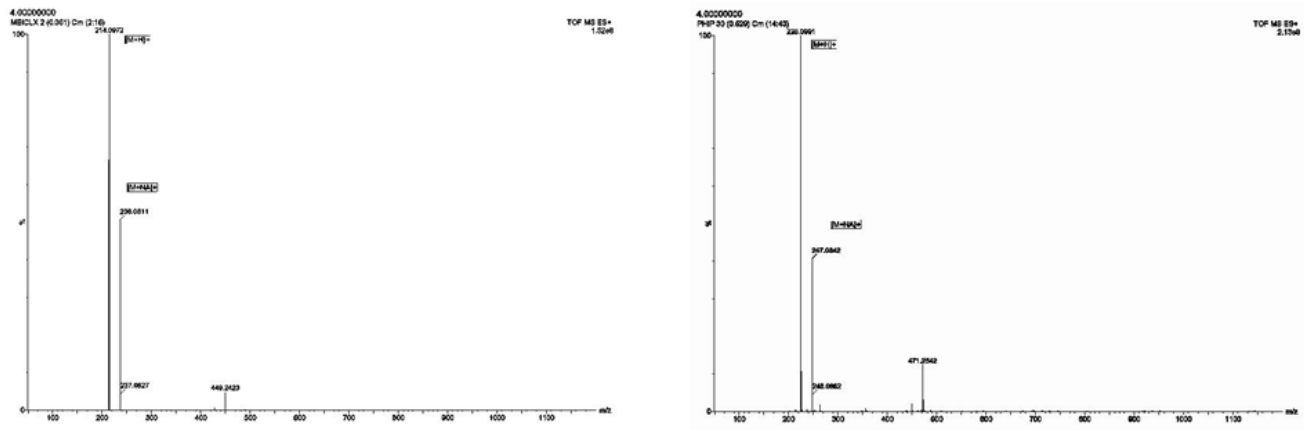


Figure 2. ESIMS of standard mutagen: a, PhIP; b,MeIQx

Table 2. The contents of fat, protein, carbohydrates, energy, in the samples analyzed

Sample and Nutritional Information		
Peppered Mackerel		
Typical Values	**Per 100 gm**	**Per Can**
Energy	1105KJ/267Kcal	1228 KJ/297Kcal
Protein	15.7 gram	14.1
Carbohydrate	Trace	Trace
(of which sugars)	Trace	Trace
Fat	26.0 gram	23.4 gram
(Saturated)	4.1 gram	3.7 gram
PUFA	6.5 grams	5.9 gram
(ω-3 PUFA)	1.4 grams	1.3 gram
Fibre	0.2 gram	0.2 gram
Sodium	0.2 gram	0.2 gram
Bacon Grill		
Typical Values	**Per 100 gram**	
Energy	1380 KJ/ 325 Kcal	
Protein	11.0 grams	
Carbohydrates	7.5 grams	
Fat	28 grams	
Chicken Salami		
Nutritional Information	**Per 100 gram**	
Energy Value	164.17 Kcal	
Protein (N x 5.8)	14.77 grams	
Fat	11.61 grams	
Saturated Fat	4.3 grams	
Trans Fatty acid	0.1 grams	
Carbohydrates	1.1 grams	
Cholesterol	64.6 mgs	
Moisture	71.54 grams	
Ash	1.93	

3.3. ESI MS for Standard Reference Mutagens

Figure 2 represents the ESI MS chromatograms of the two standard known reference mutagens employed for this study namely MeIQx and PhIP. Results obtained for Figure 2a indicates a peak at an (m/z = 214.0972), which corresponds to MeIQx for which (m/z = 213), an additional peak is obtained at an (m/z =236.08),because of the formation of a quasimolecular ion [M+Na]+, Figure 2 b indicates a prominent peak which was obtained at an (m/z = 225.0991) which corresponds to PhIP for which (m/z = 224). An additional peak at an (m/z = 247.084) was observed, which we interpret to be that of [M+Na]+ as an added sodium ion.

3.4. Identification of Mutagens through ESI MS analysis

Commercially available, sample meat products contain a number of additives, preservatives, binders and ingredients. Peppered Mackerel, Bacon Grill and Chicken Salami are ready to eat packed food products. The main ingredients and nutritional information of the experimental food items which we employed are enlisted in Table 1. Peppered Mackerel does not contain artificial flavours and colour, whereas Bacon Grill has smoke flavour as has been emphasized in the nutritional information. In order to investigate the presence of the representative mutagens in our samples under study: Bacon Grill, Peppered Mackerel and Chicken Salami, when subjected to ESI MS analysis, a distinct peak was observed for one of the samples (Bacon Grill) at an (m/z = 214.0908) which confirms the presence of MeIQx (m/z = 214.0972). We can safely conclude that Bacon Grill has a substantial amount of MeIQx, represented in Figure 3.

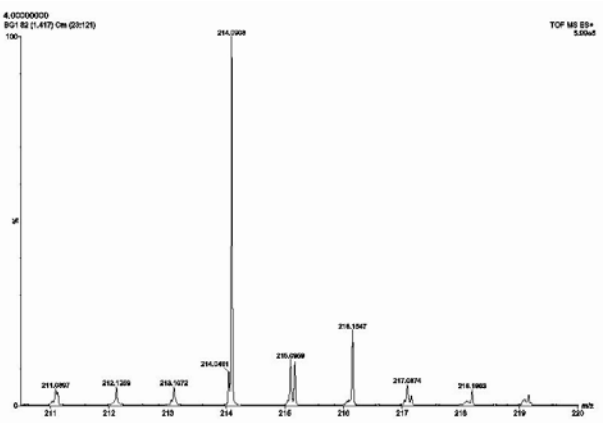


Figure 3. ESIMS chromatogram of Bacon Grill

However, when Chicken Salami (Figure 4) and Peppered Mackerel (Figure 5) were subjected to analysis through the same process, we were unable to detect the presence of any such carcinogenic compound. Further, it was of interest that in Peppered Mackerel and Chicken Salami, we were able to identify a well characterized (unassigned) chromatographic peak at an (m/z = 413), which we presume to be that of a representative of a suspected polymer species being formed at the mass represented.

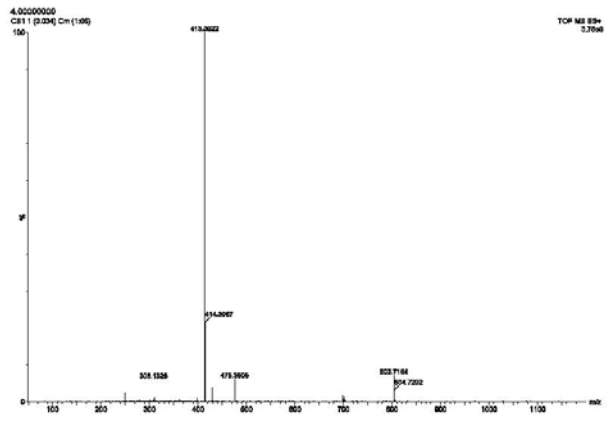


Figure 4. ESIMS chromatogram of Chicken Salami

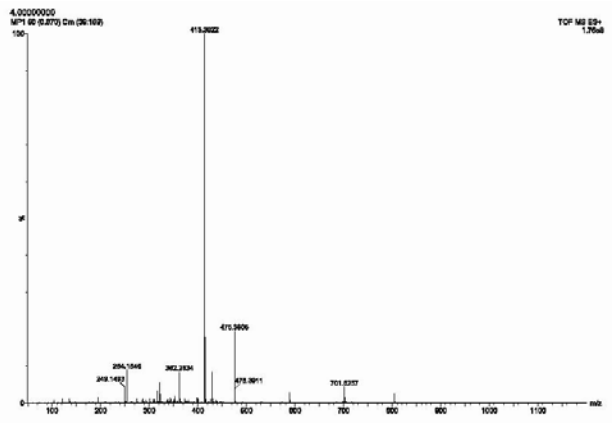


Figure 5. ESIMS chromatogram of Peppered Mackerel

4. Discussion

Carcinogenic and mutagenic heterocyclic aromatic amines are natural products often present at ng/g levels in muscle meats when cooked at temperatures above 150^{O}C. Many heterocyclic aromatic amines have been isolated and identified from cooked meats or model systems, but four compounds are frequently reported in food surveys. These are DiMeIQx (2 amino-3,4,8-trimethylimidazo[4,5-f] quinoxaline), IQ (2- amino-3-methylimidazo [4,5-f]quinoline), MeIQx (2-amino-3,8-dimethylimidazo[4,5f]quinoxaline) and PhIP (2-amino-1-methyl-6phenylimidazo[4,5-b] pyridine [8]. PhIP has been shown to have biological properties that appear to be relevant to risk assessment, more importantly tissue specificity with respect to carcinogenicity of colon in male rats and mammary gland and colon in females. Similar cancer targets have been observed in humans exposed to a diet rich in meat, suggesting a role of these compounds in human cancer etiology which necessitates an understanding of the levels of consumption of PhIP and related compounds in our diet. Human populations are exposed to mutagenic/carcinogenic HAAs mostly through consumption of meat and meat products [21,22]. Further, it was observed that MeIQx and PhIP were reported to occur more frequently [23].

To summarize this study, HAAs were determined in three different types of samples obtained from local grocery stores. The validated method provided accurate results, constituting a simple and easy method to identify the presence of such carcinogenic/mutagenic compounds in foods commonly consumed by humans. Among the three samples analyzed by us, a positive result was obtained for one of the samples, namely Bacon Grill. We propose that a higher fat content (24.6%) in pork may facilitate the formation of HAAs through Maillard reaction products such as pyridine from lipid degradation. Although pepperd mackerel also has a high fat content, it has black pepper as one of the ingredients to which may presumably be attributed the inhibition of generation of Heterocyclic Aromatic Amines as has been shown by previous workers [24].

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Statement of Competing Interests

The authors disclose there is no conflict of interest. No competing financial interests exist.

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