

## Identification of Heterocyclic Amines in Indian Home Cooked and Commercially Available Meat Foods

Rana Zaidi\* and Pinki Rani Rawat

Department of Biochemistry, Faculty of Science, Jamia Hamdard University, New Delhi 110062, India

### Abstract

Some typical Indian cooked / fried meat dishes like kababs, nuggets and industrially prepared meat foods were analysed for the content of two potentially carcinogenic heterocyclic amines PhIP and MeIQx. The total amount of PhIP and MeIQx detected was highest (325.83 ng / g) in fried fish whereas the total amount of PhIP and MeIQx detected was lowest in chicken nuggets (166.44 ng/g). Identification of these food mutagens was done by Gas Chromatography and Mass Spectrometry. The results of this study indicate that the content of heterocyclic amines in a particular dish may vary with origin. Methods of cooking meat should be suitably modified to minimise the risk of exposure to PhIP and MeIQx.

**Keywords:** Fast food; Fried food; Heterocyclic amines; Meat mutagens; Carcinogens

**Abbreviations:** PhIP: 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine; CAS no: 105650-23-5; MeIQx: 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline; CAS no: 77500-04-0

### Introduction

Heterocyclic amines (HAS) belong to a group of mutagenic / carcinogenic compounds formed during cooking of foods, especially muscle meat. Since some heterocyclic amines are considered to be possible human carcinogens much work has been devoted to clarify their role in the etiology of cancer. Several epidemiological studies have shown high meat consumption to be associated with an increased risk for cancer. Despite some recently published data about the amounts of HAS in fast food meat products and commercially available cooked foods [1,2] there is still a lack of information to establish a sufficient analytical base for risk evaluation.

Eating habits are changing all over the world due to change in life style and there is an increased tendency to rely on ready to eat foods produced by the food industry and on fast food chains and restaurants. As food preparation practices and dietary habits differ among countries, the amounts of HAS in cooked / fried meat vary with cooking conditions, meat type, shape of the product [3]. It may also be influenced by the recipes for example, addition of common salt, sugar, potato starch and spices [4-8].

Other factors affecting the formation of HAS are temperature and duration of cooking, cooking strategies, acidity, type of animal flesh, amino acid present, type of cooking oil used, fat content and the amount of precursors present in meat [9-14]. Frying or grilling has been found to produce higher amounts of heterocyclic aromatic amines than do indirect heat methods [15-18]. Deep frying in a large volume of vegetable oil generates higher amounts of such mutagens [13]. Several studies have shown production of these heterocyclic amines in commercially available food items [11,19-21].

Marinades, brine or seasoning that contain antioxidants may reduce formation of HAS [1,6]. Not much has been reported on the contents of HAS in food from restaurants and fast food outlets, ready to eat meat foods manufactured by the food industry and fried meat foods [2, 22-26].

The objective of this study, therefore, was to estimate the amounts of PhIP and MeIQx in traditional Indian fried meat foods, ready to eat commercial foods and fast foods. Identification and quantification of heterocyclic amines was carried out by reverse-phase UPLC with photodiode array detector, Gas Chromatography of the Perfluoropropionic acid derivatives was performed to confirm the results. Further samples were analysed by Mass Spectrometry to reconfirm the results. The significance of this study lies in furthering our understanding of risk assessment and cancer chemoprevention in future.

### Materials and Methods

#### 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, sodium chloride, acetone were obtained from S.D.Fine Chemicals (Mumbai, India); methanol, benzene, dimethylsulphoxide were purchased from Sisco Research Laboratories (Mumbai, India); ammonia solution from Merck (Mumbai, India); triethylamine, tris buffer from Spectrochem (Mumbai, India). All reagents were analytical grade. For derivatization pentafluoropropionic anhydride was obtained from Sigma Aldrich (Saint Louis, USA).

Reagents for UPLC analysis were HPLC grade, acetonitrile, methanol, trifluoroacetic acid were purchased from Merck (Mumbai,

\*Corresponding author: Rana Zaidi, Department of Biochemistry, Faculty of Science, Jamia Hamdard University, New Delhi 110062 India, Tel: +091-11-26059688; E-mail: [ranaamu@yahoo.com](mailto:ranaamu@yahoo.com)

Received July 22, 2011; Accepted August 29, 2011; Published September 19, 2011

**Citation:** Zaidi R, Rawat PR (2011) Identification of Heterocyclic Amines in Indian Home Cooked and Commercially Available Meat Foods. J Nutr Food Sci 1:107. doi:10.4172/2155-9600.1000107

**Copyright:** © 2011 Zaidi R, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

India). The mobile phase was filtered through 0.45 µm nylon filters (Millipore, Bangalore, India). The extracts were also filtered before injecting through 0.45 µm syringe filter from AxivaSichem Biotech (Delhi, India).

### Sample preparation

All the raw boneless meat samples (beef, mutton, chicken and fish) and commercial foods (chicken kabab, chicken nugget, sardine in tomato sauce and sardine in olive oil) were purchased from local markets in New Delhi. They were either deep fried in a large volume of cooking oil or used directly, as follows:

(a) Deep frying: 50 g of each boneless sample was deep fried in commercially available Dhara refined mustard oil on a high flame in a kadhai (Indian deep frying pan) for 20 minutes.

(b) Commercially available meat foods: 50 g of Chicken kabab and Chicken nuggets were deep fried for approximately 20 minutes in Dhara refined mustard oil while sardine in olive oil and sardine in tomato sauce (canned products) were used as such for our studies.

All samples were stored at -20°C until analysed.

### Extraction of Heterocyclic amines from meat samples

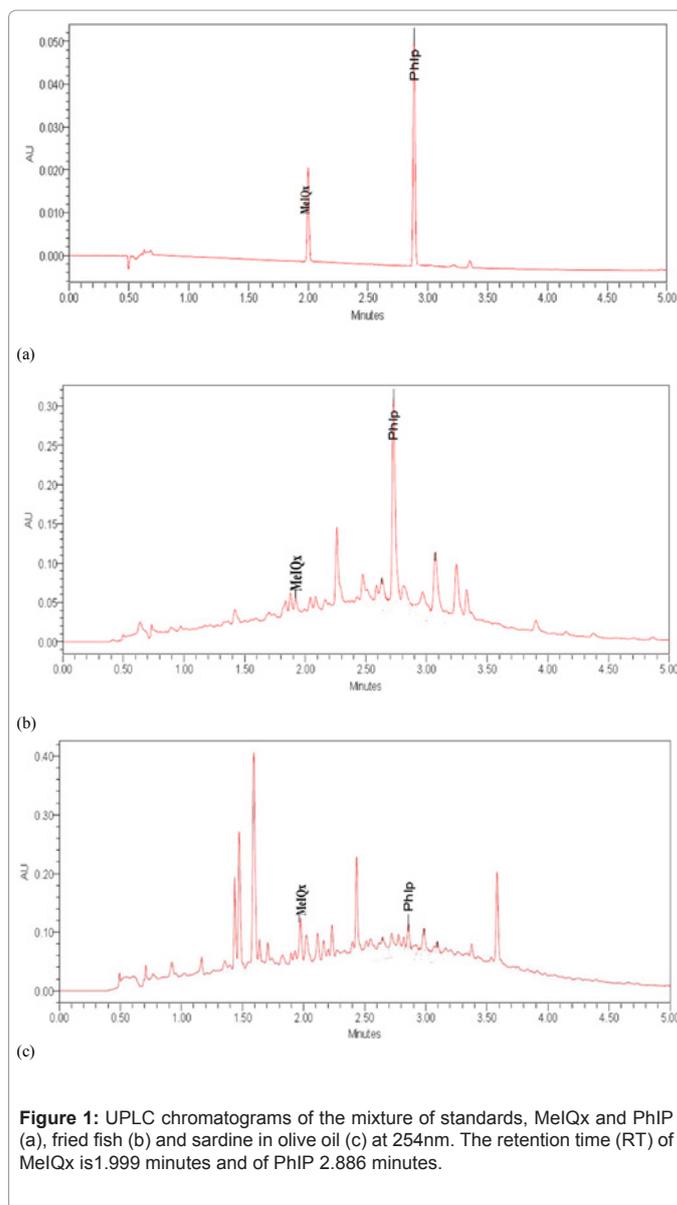
Mutagens were extracted according to the method of Bjeldanes et al. [27] with certain modifications [13] consisting of a liquid-liquid extraction procedure at different pH followed by a solid-liquid extraction on Amberlite XAD-2 resin. 50 g of meat sample was taken each time and homogenised in a double 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 was filtered through Whatman filter paper no.1 and clear filtrate applied to a column of Amberlite XAD-2 resin (1.5cm×10cm) at 2 ml/min. Then 10 ml of distilled water (pH 7.0) was introduced to the column and the adsorbed compounds finally eluted with 25 ml of acetone followed by 25 ml of methanol. Extracts were evaporated to dryness in a vacuum rotary evaporator and resuspended in 1ml methanol to be used for UPLC and GC.

### Ultra performance liquid chromatography (UPLC)

Chromatographic separation of HAS was performed on a Waters Acquity ultra performance liquid chromatography system (Milford, MA, USA) equipped with a binary solvent manager, an auto sampler, column manager composed of a column oven, a precolumn heater and a photo diode array detector. Five microliter of final analytical solution

Meat source	Main Ingredients
Sardines in tomato sauce	120 grams total weight, 84 grams drained weight, sardines, water, concentrated tomato puree water.
Sardines in olive oil	120 grams total weight, 90 grams drained weight, olive oil, salt
Chicken nuggets	Chicken meat 52.6%, bread crumbs wheat flour, yeast, salt permitted bread improvers Class II preservative calcium propionate (E 282) acetic acid (E260), water, edible vegetable oil batter, wheat flour, corn flour, corn starch, salt emulsifier sodium carboxymethylcellulose (E 466), iodised salt, soya protein and phosphate.
Chicken kabab	Chicken meat, onion, garlic, green chillies, salt.

**Table 1:** Main ingredients of the commercial food sources selected for this study.



**Figure 1:** UPLC chromatograms of the mixture of standards, MelQx and PhIP (a), fried fish (b) and sardine in olive oil (c) at 254nm. The retention time (RT) of MelQx is 1.999 minutes and of PhIP 2.886 minutes.

(a)			
Name of the meat source	Amount of MelQx	Amount of PhIP	Total HAS (MelQx + PhIP)
Fried beef	4.97	27.64	32.61
Fried mutton	22.02	19.77	41.79
Fried chicken	42.91	15.48	58.39
Fried fish	31.97	293.86	325.83
(b)			
Name of the meat source	Amount of MelQx	Amount of PhIP	Total HAS (MelQx + PhIP)
Sardine in tomato sauce	37.46	23.64	61.10
Sardine in olive oil	18.74	28.59	47.33
Chicken nuggets	135.77	30.67	166.44
Chicken kabab	11.99	155.04	167.03

**Table 2:** Quantification (ng/g) of MelQx and PhIP in fried (a) and commercial products (b) by UPLC.

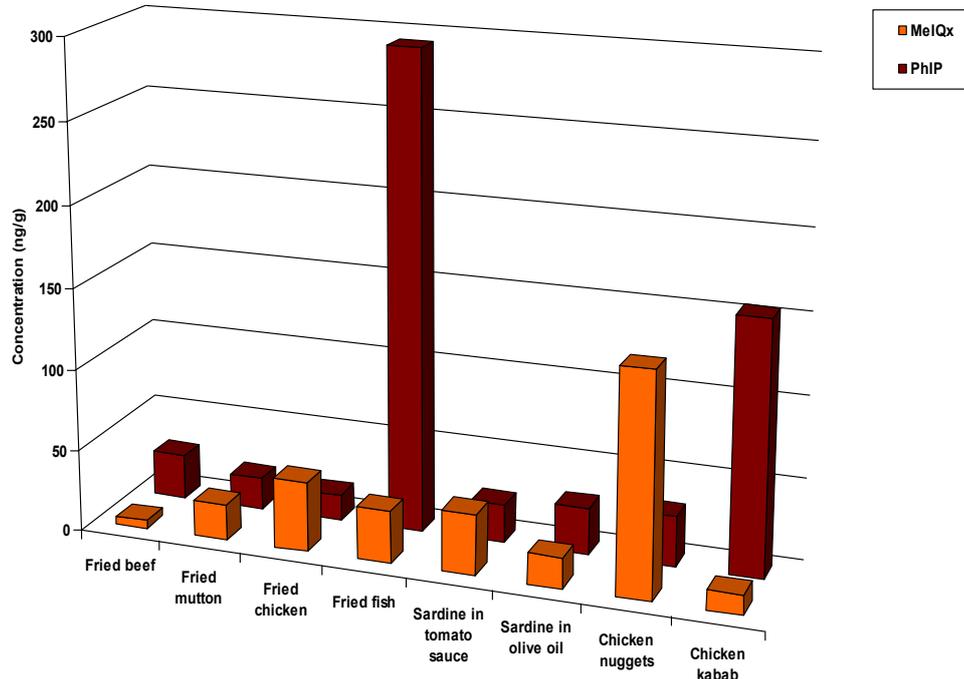


Figure 2: Bar graph illustrating the amount (ng/g) of MeIQx and PhIP in the selected deep fried and commercial meat sources as detected by UPLC.

was injected into an ACQUITY UPLC<sup>®</sup> HSS T3 column (100 mm × 2.1 mm id., 1.7 μm particle size). UPLC column was kept at 50°C and the chromatographic separation was performed by gradient elution. The mobile phase consisted of a mixture of A: 0.1 % trifluoroacetic acid (TFA) in water and B: acetonitrile, and the flow rate of 0.5 ml/min was employed.

The gradient elution programme was 0-1.32 min, 25% B; 1.32-2.76 min, 25-55% B; 2.76-3.38 min, 55-75% B; 3.38-4.5 min, 75-10% B and then 4.5-5.5 min, 10% B and equilibration of the column. The gradient analysis was performed at 263 nm wavelength and at 315 nm for MeIQx and PhIP respectively. Data acquisition, data handling and instrument control were performed by Empower 2154 Software v1.0. All extracts were filtered through 0.45 μm filter prior to injection in the UPLC column. Quantification was performed by external calibration curve method.

**Standard Calibration curves for the two heterocyclic amines evaluated:** 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. Both MeIQx and PhIP were prepared in concentrations of 3, 6 and 9 ng/ml. Detection of MeIQx and PhIP was performed at 263 nm and 315 nm wavelength respectively. The method was found to be selective, linear (correlation coefficient for MeIQx,  $r^2 = 0.99913$  and for PhIP,  $r^2 = 0.999202$ ).

#### Gas chromatography (GC)

PhIP and MeIQx are polar and non volatile, therefore they tend to elute as broad and tailing peaks due to their strong adsorption to the column and injector during GC analysis. To overcome this problem the HAS were first derivatized to amides by acylation reaction with pentafluoropropionic anhydride (PFPA).

**Derivatization of Heterocyclic amines:** Each sample was dissolved in a volume of 500 μl of benzene in a 5 ml vial. Subsequently, a 100 μl of 0.05M triethylamine in benzene and 10 μl of pentafluoropropionic anhydride (PFPA) were added. The vials were capped and heated at 50°C for 15 minutes and then cooled to room temperature, now a 1 ml 5% aqueous ammonia was added to it. The solution was allowed to mix for 5 minutes and centrifuged at high speed for about 5 minutes to separate the upper benzene layer. 2 μl of this benzene layer was injected into the GC column for analysis.

**Instrumentation conditions for Gas Chromatography:** GC analysis of the PFPA derivatives was performed on a Shimadzu GC-2010 gas chromatograph equipped with an AOC-20i Auto injector and a flame ionization detector (FID). 2 μl of the benzene layer was injected onto an Rtx 1MS column (30 m × 0.25 mm id., film thickness 0.25 μm) in a splitless injection mode. N<sub>2</sub>/Air was used as carrier gas at 1.2 ml/min flow rate. Injection temperature was 270°C while detector temperature was 280°C. The column oven temperature program was as follows: initial column oven temperature was 100°C held for 1 minute; gradually the column temperature was increased from 100°C to 250°C at 18°C/min and held for 2 minutes, then from 250°C to 300°C at 22°C/min held for 11 minutes. Total run time was 35.25 minutes. Make-up gas flow-rate was 30 ml/min. Analysis of the chromatogram was acquired by GC solution software.

#### Matrix assisted laser desorption/ionization-Time of flight-Mass Spectrometry (MALDI-TOF-MS)

MALDI-MS was performed on a Bruker Daltonics Autoflex II TOF instrument. 1 μl of the sample was mixed with the matrix solution, 20 mg/ml sinnapinic acid in acetonitrile: water: TFA (50:50:0.1) and applied onto the α-cyano-4-hydroxycinnamic acid matrix. The laser (Nitrogen UV, 337 nm) was fired at the matrix which vaporizes the samples. At the same time a voltage of 17-19 kV was applied to the

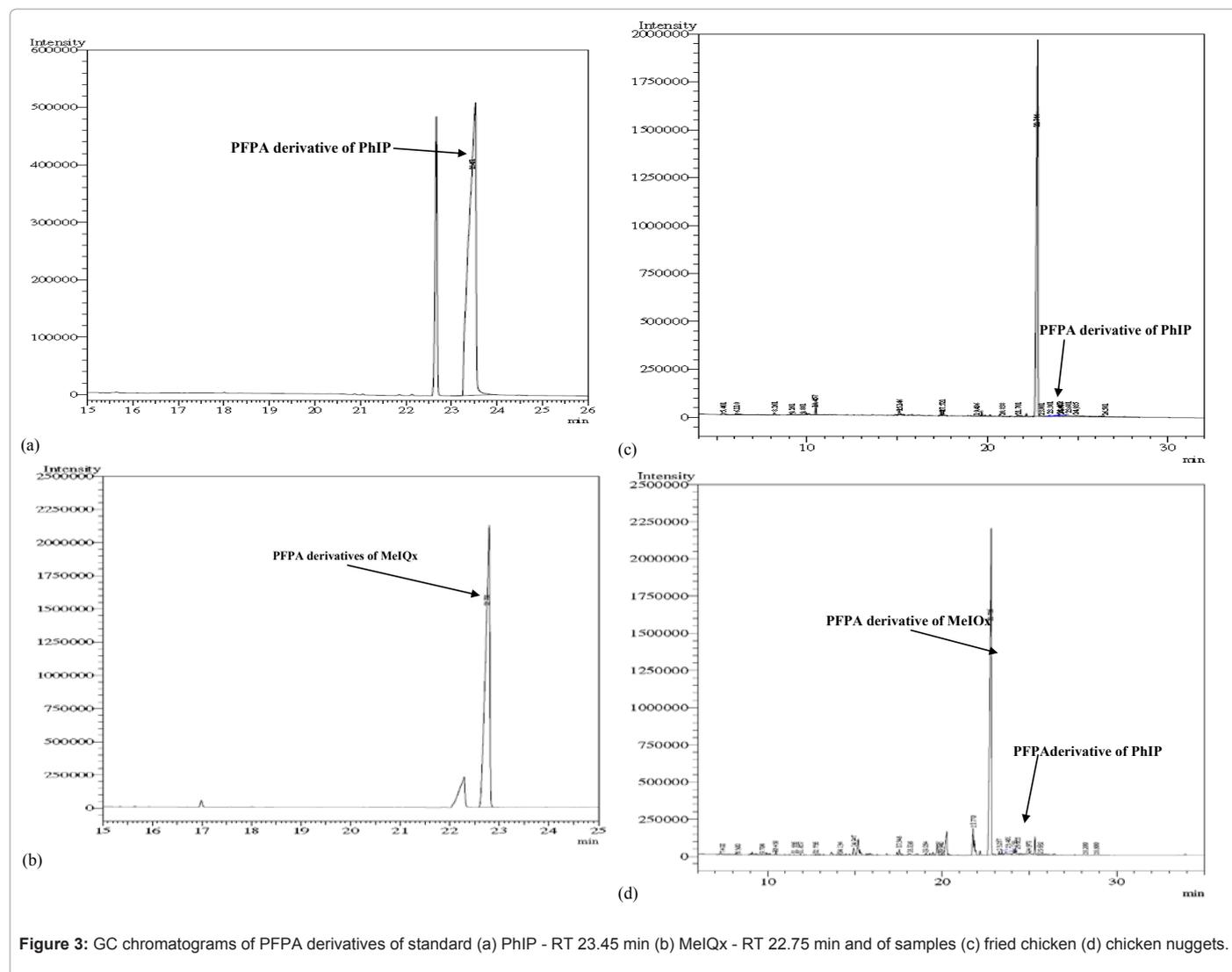


Figure 3: GC chromatograms of PFPA derivatives of standard (a) PhIP - RT 23.45 min (b) MeIQx - RT 22.75 min and of samples (c) fried chicken (d) chicken nuggets.

target plate to accelerate the ionised sample towards a time of flight mass analyser. Analysis of Mass spectra was acquired using Flex control and Flex analysis software and a graph was plotted against intensity and m/z ratio.

### Statistical analysis

The t-test was performed employing the Graph Pad software for analysing statistical significance between MeIQx and PhIP in various types of home cooked and commercial samples.

### Results and Discussion

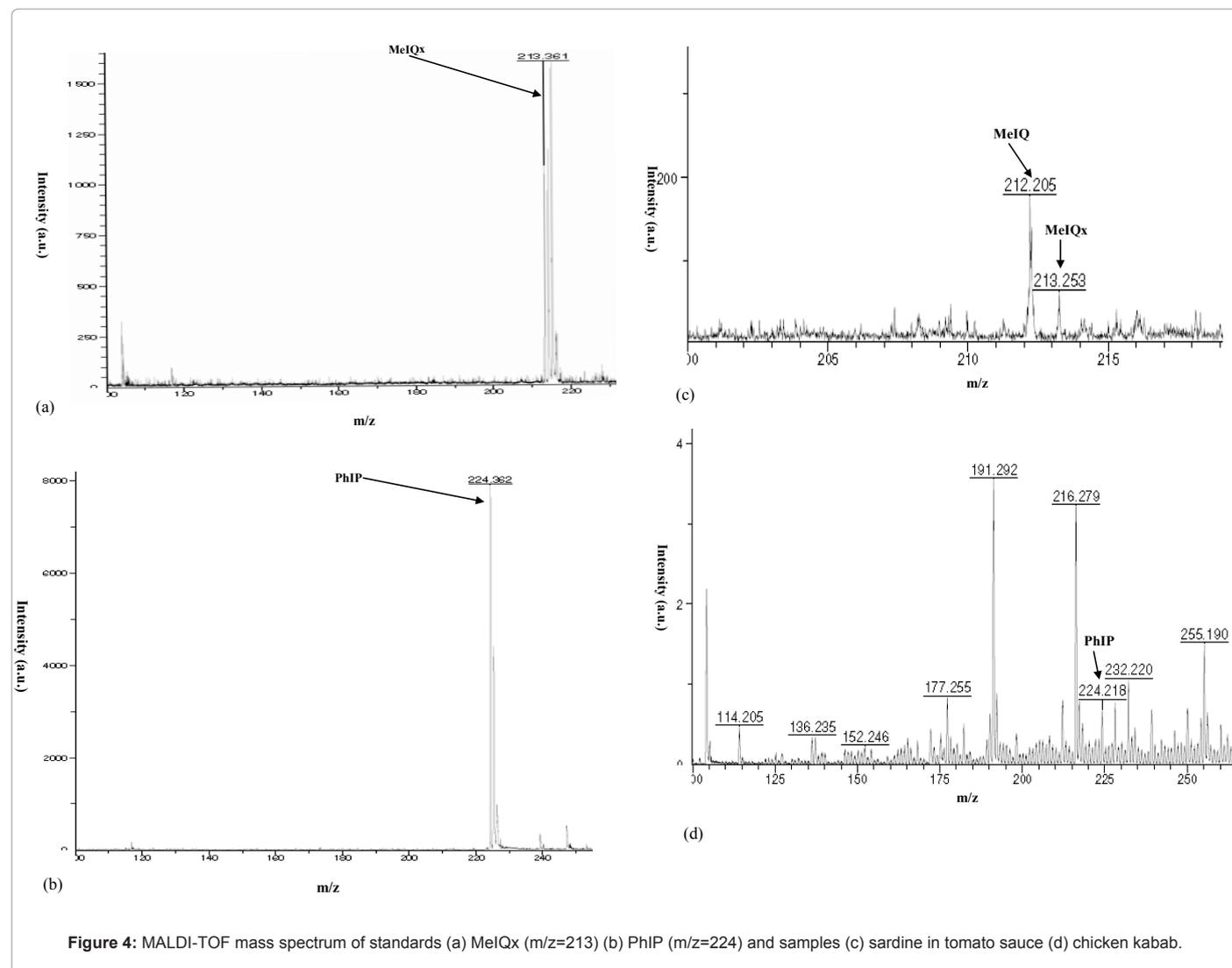
All the boneless meat samples (beef, mutton, chicken and fish) were shredded into small pieces and deep fried on medium flame for 20 minutes in mustard oil before processing. The commercial meat products contain a number of spices and preservatives as mentioned in material and methods. Sardine samples (sardines in tomato sauce and sardine in olive oil) are ready to eat, these are tinned products although chicken kabab and chicken nuggets need to be deep fried thoroughly before consumption. The main ingredients of these food items are listed in Table 1. The UPLC chromatograms of the standard mixture (MeIQx&PhIP), fried fish and sardine in olive oil are illustrated

in Figure 1. Figure 1, panel a shows the UPLC chromatograms of standard known mutagens: MeIQx and PhIP. Results indicate that the retention time for MeIQx is 1.999 minutes, for PhIP it is 2.886 minutes respectively. Panel 1b is the chromatogram obtained for deep fried fish, showing formation of both the species, MeIQx and PhIP. When sardine in olive oil (1c) was analysed, both MeIQx and PhIP were found to be formed. We can safely conclude that both MeIQx and PhIP are formed in the mentioned samples.

Figure 2 is the bar graph depicting the amounts in (ng/g) of both dietary mutagens MeIQx and PhIP detected in all the eight samples which we selected for our study. Among all of the commercial samples analysed, maximum amount of PhIP was observed to be formed in chicken kabab, whereas a maximum amount of MeIQx was detected in chicken nuggets. When the home cooked samples were analysed, fried fish was able to generate maximum amount of PhIP, fried chicken was able to produce a maximum of MeIQx.

### Effect of deep frying on the generation of PhIP and MeIQx

Heterocyclic amines in all of the samples were quantified by Ultraperformance Liquid Chromatography and the results are



illustrated in Table 2 and Figure 2, amounts are analysed in ng/g. Results suggest that beef and fish produce PhIP in higher amounts while mutton and chicken generate higher amounts of MeIQx [28-30]. Among all the deep fried samples analysed, fish was found to produce the highest amount of PhIP (293.86ng/g) while chicken showed the maximum production of MeIQx (42.9ng/g). In beef we observed a higher production of PhIP (27.64ng/g) as compared to MeIQx (4.97ng/g). These amounts are similar to that obtained by previous studies (31ng/g PhIP and 7ng/g MeIQx)[31].

In deep fried chicken we observed more of MeIQx(42.91ng/g) as compared to PhIP (15.48ng/g) whereas others [32] (38.2ng/g PhIP and 1.8ng/g MeIQx) and [33] (48.54ng/g PhIP and 2.34ng/g MeIQx) reported the opposite results upon pan frying these samples. Fish when subjected to deep frying generated the highest amount of PhIP (293.86ng/g) whereas MeIQx was found to be 31.97ng/g as reported previously [34] PhIP (69.2ng/g) and MeIQx (6.44ng/g) in fish on pan frying at 260°C for 16 minutes.

Alternatively, mutton samples when subjected to the same treatment were also found to generate significant amounts of PhIP

(19.77ng/g) and MeIQx (22.02ng/g).

#### Commercial/ready to eat/tinned meat foods

Among the four commercial samples which we analysed, sardine in tomato sauce was observed to have the least amount of the total heterocyclic aromatic amines (47.33ng/g) with 28.59ng/g PhIP and 18.74ng/g of MeIQx respectively. Previous studies [22,35] detected 16.6ng/g MeIQx upon broiling and grilling sardine respectively. In chicken nuggets MeIQx was found to be 135.77ng/g and PhIP 30.67ng/g, whereas in chicken kabab, MeIQx was detected to be 11.99ng/g, PhIP was 155.04ng/g.

Among the fried samples which were analysed the highest amount of total heterocyclic amines (MeIQx + PhIP) were observed to be produced in fried fish (325.83ng/g) followed by fried chicken (58.39ng/g) fried mutton (41.79ng/g) and fried beef (32.61ng/g). On the other hand, among the commercial food products analysed the order of generation of meat mutagens gradually decreased from maximum to minimum amounts in the order mentioned: chicken kabab (167.03ng/g), chicken nuggets (166.44ng/g), sardine in olive oil

(61.10ng/g) and sardine in tomato sauce (47.33ng/g) respectively. Our results for sardines in olive oil and sardines in tomato sauce are clearly suggestive of the fact that marinades, brine or seasoning that contain antioxidants may reduce the formation of HAS [1,6]

### Statistical significance

The Paired t test for significant differences between MeIQx and PhIP in various types of home cooked and commercial samples was performed and it gave a two tailed p value for MeIQx = 0.3820, considered non significant, whereas the two tailed p value for PhIP = 0.4764, was also considered non significant.

### Identification of meat mutagens through Gas Chromatography and Mass Spectrometry

Perfluoropropionic acid derivatives were prepared as indicated in the methodology and analysed through gas chromatography and mass spectrometry to reconfirm the results. Derivatives of PhIP and MeIQx were identified in standard reference samples which is illustrated in Figure 3 a and 3b as well as in fried chicken (Figure 3c) and chicken nuggets (Figure 3d). Figure 3 are the gas chromatograms which demonstrate the PFFA derivative of a) PhIP in which the retention time of the derivatised sample is 23.45 minutes. Panel b is derivatised MeIQx for which the retention time is 22.75 minutes. Figure 3c shows the GC analysis of fried chicken, we observe the PFFA derivative of MeIQx, retention time= 22.75 minutes. This supports our results of Figure 2, this result further authenticates the production of MeIQx in this sample. Figure 3d demonstrates the detection of MeIQx in chicken nuggets for which retention time=22.75 minutes. PFFA derivative of PhIP is also observed in the graph, thereby proving the formation of MeIQx and PhIP in chicken nuggets.

Figure 4 shows the MALDI-TOF mass spectrum of standard mutagens: MeIQx (m/z = 213), for PhIP (m/z = 224). Panel a is the mass spectrum of the standard reference mutagen MeIQx, we observe a prominent peak at an m/z value of 213. Panel 4 b indicates a single peak in this sample at m/z = 224, confirming the detection of PhIP. When sardine in tomato sauce was subjected to mass spectrometry we observed a peak at an m/z=213, thereby proving the detection of MeIQx in this sample. An additional peak at m/z=212 has been detected, which corresponds to another known meat mutagen : 2-amino-3,4-dimethylimidazo[4,5-f] quinoline or MeIQ. Panel 4d shows the mass spectrum of chicken kabab. Results show a peak obtained at m/z=224 which corresponds to PhIP detected in this commercial sample.

### Concluding Remarks

Risk assessment for cancer prevention is necessary to increase our knowledge on levels of exposure to HAS. Our study shows that the amounts of HAS in home cooked and commercial ready to eat home fried meat products are generally very high, but some foods may have low amounts. Indian cooking conditions, ingredients, spices and antioxidants play an important role in the amounts and production level of HAS.

### Acknowledgements

University Grants Commission, New Delhi is duly acknowledged for providing financial assistance for the major research project UGC-MRP- F.No. 33-221/2007(SR) on which this paper is based. Ajai Kumar is acknowledged for technical assistance in Gas Chromatography.

### References

1. Salmon CP, Knize MG, Felton JS (1997) Effects of marinating on heterocyclic amine carcinogen formation in grilled chicken. *Food Chem Toxicol* 35: 433-441.
2. Knize MG, Sinha R, Brown ED, Salmon CP, Levander OA, et al. (1998) Heterocyclic Amine Content in Restaurant-cooked Hamburgers, Steaks, Ribs and Chicken. *J Agric Food Chem* 46: 4648-4651.
3. Skog KI, Johansson MA, Jagerstad MI (1998) Carcinogenic heterocyclic amines in model systems and cooked foods: a review on formation, occurrence and intake. *Food Chem Toxicol* 36: 879-896.
4. Persson E, Sjöholm I, Skog K (2003) Effect of high water holding capacity on the formation of heterocyclic amines in fried beef burgers. *J Agric Food Chem* 51: 4472-4477.
5. Gu YS, Kim IS, Park JH, Lee SH, Park DC, et al. (2001) Effects of seasoning and heating device on mutagenicity and heterocyclic amines in cooked beef. *Biosci Biotechnol Biochem* 65: 2284-2287.
6. Murkovic M, Steinberger D, Pfannhauser W (1998) Antioxidant spices reduce the formation of heterocyclic amines in fried meat. *Z. Lebensm. \_ Unters. \_ Forsch. A* 207: 477-480.
7. Skog K, Jagerstad M (1990) Effect of monosaccharides and disaccharides on the formation of food mutagens in model systems. *Mutat Res* 230: 263-272.
8. Wang YY, Vuolo LL, Spingarn NE, Weisberger JH (1982) Formation of mutagens in cooked foods. V. The mutagen reducing effect of soy protein concentrates and antioxidants during frying of beef. *Cancer Lett* 16: 179-189.
9. Bjeldanes LF, Morris MM, Timourian H, Hatch FT (1983) Effects of meat composition and cooking conditions on mutagen formation in fried ground beef. *J Agric Food Chem* 31: 18-21.
10. Davies JE, Chipman JK, Cooke MA (1993) Mutagen formation in beef burgers processed by frying or microwave with use of flavouring and browning agents. *J Food Sci* 58: 1216-1223.
11. Lan CM, Chen BH (2002) Effects of soy sauce and sugar on the formation of heterocyclic amines in marinated foods. *Food Chem Toxicol* 40: 989-1000.
12. Pariza MW, Ashoor SH, Chu FS, Lund DB (1979) Effects of temperature and time on mutagen formation in pan fried Hamburger. *Cancer Lett* 7: 63-69.
13. Perez C, Lopez De Cerain A, Bello J (2002) Modulation of mutagenic activity in meat samples after deep-frying in vegetable oils. *Mutagenesis* 17: 63-66.
14. Johansson M, Jägerstad M (1993) Influence of oxidized deep-frying fat and iron on the formation of food mutagens in a model system. *Food Chem Toxicol* 31: 971-979.
15. Layton DW, Bogen KT, Knize MG, Hatch FT, Johnson VM, et al. (1995) Cancer risk of heterocyclic amines in cooked foods: an analysis and implications for research. *Carcinogenesis* 16: 39-52.
16. Starvic B (1994) Biological significance of trace levels of mutagenic heterocyclic aromatic amines in human diet: a critical review. *Food Chem Toxicol* 32: 977-994.
17. Skog K (1993) Cooking procedures and food mutagens: a literature review. *Food Chem Toxicol* 31: 655-675.
18. Keating GA, Bogen KT (2001) Methods for estimating heterocyclic amine concentrations in cooked meats in the US diet. *Food Chem Toxicol* 39: 29-43.
19. Richling E, Kleinschnitz M, Schreier P (1999) Analysis of heterocyclic aromatic amines by high resolution gas chromatography-mass spectrometry: a suitable technique for the routine control of food and process flavours. *Eur Food Res Technol* 210: 68-72.
20. Richling E, Haring D, Herderich M, Schreier P (1998) Determination of Heterocyclic Aromatic Amines (HAA) in Commercially Available Meat Products and Fish by High Performance Liquid Chromatography-Electrospray Tandem Mass Spectrometry (HPLC-ESI/MS). *Chromatographia* 48: 258-262.
21. Borgen E, Skog K (2004) Heterocyclic amines in some Swedish cooked foods industrially prepared or from fast food outlets and restaurants. *Mol Nutr Food Res* 48: 292 - 298.
22. Keating GA, Sinha R, Layton D, Salmon CP, Knize MG, et al. (2000) Comparison of heterocyclic amine levels in home-cooked meats with exposure indicators (United States). *Cancer Causes Control* 11: 731-739.

23. Tikkanen LM, Sauri TM, Latva-Kala KJ (1993) Screening of heat – processed Finnish foods for the mutagens 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline, 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine. *Food Chem Toxicol* 31: 717-721.
24. Johansson M, Jägerstad M (1994) Occurrence of mutagenic / carcinogenic heterocyclic amines in meat and fish products, including pan residues, prepared under domestic conditions. *Carcinogenesis* 15: 1511-1518.
25. Knize MG, Sinha R, Rothman N, Brown ED, Salmon CP, et al. (1995) Heterocyclic amine content in fast food meat products. *Food Chem Toxicol* 33: 545-551.
26. Holder CL, Cooper WM, Churchwell MI, Doerge DR, Thompson S (1996) Multi-residue determination and confirmation of ten heterocyclic amines in cooked meats. *J Muscle Foods* 7:281-290.
27. Bjeldanes LF, Grose KR, Davis PH, Stuermer DH, Healy SK, et al. (1982) An XAD-2 resin method for efficient extraction of mutagens from fried ground beef. *Mutat Res* 105: 43-49.
28. Turesky RJ, Taylor J, Schnackenberg L, Freeman JP, Holland RD (2005) Quantitation of carcinogenic heterocyclic aromatic amines and detection of novel heterocyclic aromatic amines in cooked meats and grill scrapings by HPLC/ESI-MS. *J Agric Food Chem* 53: 3248-3258.
29. Murray S, Lynch AM, Knize MG, Gooderham NJ (1993) Quantification of the carcinogens 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline, 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine in food using a combined assay on capillary column gas chromatography negative ion mass spectrometry. *J. Chromatogr (Biomedical Application)* 616: 211-219.
30. Wakabayashi K, Ushiyama H, Takahashi M, Nukaya H, Kim SB, et al. (1993) Exposure to heterocyclic amines. *Environ Health Perspectives* 99: 129-134.
31. Knize MG, Dolbear FA, Carroll KL, Moore DH II, Felton JS (1994) Effect of cooking time and temperature on the heterocyclic amine content of fried beef patties. *Food Chem Toxicol* 32: 595-603.
32. Oz F, Kaban G, Kaya M (2010) Effects of cooking techniques and levels on the formation of heterocyclic aromatic amines in Chicken and Fish. *J Animal Veterinary Advances* 9: 1259-1264.
33. Ni W, McNaughton L, LeMaster DM, Sinha R, Turesky RJ (2008) Quantitation of 13 heterocyclic aromatic amines in cooked Beef, Pork, and Chicken by liquid chromatography- Electrospray Ionisation/Tandem mass spectrometry. *J Agric Food Chem* 56: 68-78.
34. Zhang XM, Wakabayashi K, Liu ZC, Sugimura T, Nagao M (1988) Mutagenic and carcinogenic heterocyclic amines in Chinese cooked foods. *Mutat Res.* 201: 181-188.
35. Yamaizumi Z, Kasai H, Nishimura S, Edmonds CG, McCloskey JA (1986) Stable isotope dilution quantification of mutagens in cooked foods by combined liquid chromatography-thermospray mass spectrometry. *Mutat Res.* 173: 1-7.