Pakistan J. Zool., vol. 52(6), pp 2397-2400, 2020 **DOI**: https://dx.doi.org/10.17582/journal.pjz/20190509050501

Short Communication

Determination of Heterocyclic Amines in Ready to Eat Chicken Kabab Commercially Available from Pakistani Markets

Ishrat Perveen[1], Yasar Saleem[2] and Javed Iqbal Qazi[3]*

[1]*Department of Zoology, University of the Punjab, Lahore, Pakistan*
[2]*Biotechnology and Food Research Centre, PCSIR Labs Complex, Ferozpur Road, Lahore, Pakistan*
[3]*Department of Zoology, University of the Punjab, Lahore-54600, Pakistan.*

Article Information
Received 09 May 2019
Revised 29 May 2019
Accepted 11 June 2019
Available online 21 September 2020

Authors' Contribution
IP conducted all practical and theoretical work. JIQ and YS supervised all practical work and manuscript.

Key words
Heterocyclic amines, HCAs, Meat, IQ, MeIQX, PhIP

ABSTRACT

Presence of heterocyclic amines (HCAs) in high proteinaceous food specifically in cooked meats is a point of great concern for the health risk factor of Pakistani community. Ready-to-eat (RTE) chicken kabab samples of four commercially available brands (K, S, D and B) were analysed for the simultaneous determination of HCAs *i.e.* 2-amino-3-methyl-imidazo[4,5-*f*] quinoline (IQ), 2-amino-3,8-dimethylimidazo [4,5-*f*] quinoxaline (MeIQx) and 2-amino-1-methyl-6-phenylimidazo [4,5-b] pyridine (PhIP) using HPLC-UV detector. Quantitative analysis of the control samples (only thawed at room temperature) revealed presence of HCAs in K, S, D and B brands with respective values of 0.97, 1.09, 5.63 and 13.66 µg/g. The high thermal cookings of the RTE samples enhanced the levels of HCAs. Microwave cooking was found to produce highest total HCAs contents (0.71-35 µg/g) followed by deep-frying (1.62-22.21 µg/g) and pan-frying (1.11-8.57 µg/g) of the RTE chicken kabab samples. Highest level of IQ appeared following microwave cooking (nd-35µg/g) followed by pan-frying (0.25-7.48 µg/g), deep-frying (nd-2.3 µg/g) and defrosting (nd-0.2 µg/g). It is speculated from our results that dietary intake of 40g RTE ckicken kabab /day indicates a relatively very high consumption of HCAs/day which may catch the level up to 1.4 mg/d. To the best knowledge of the authors, it is the highest known dietary consumption value of HCAs worldwide. This study presents a plausible positive association between the high temperature cooking methods and higher quantities of HCAs in RTE Chicken kabab commercially available from Pakistani markets.

Heterocyclic amines (HCAs) are carcinogenic/mutagenic compounds present in cooked proteinaceous muscle foods such as chicken, mutton and beef etc. (Shabbir *et al*., 2015). High thermal cooking of meat is considered a serious dietary risk factor for the production of heterocyclic amines (Puangsombat *et al*., 2011). They are divided in two main families which are thermic HCAs or aminoimidazo- azaarenes and Pyrolytic HCAs or amino-carbolines (Solyakov and Skog, 2002). Muscle food originally contains three main precursors of heterocyclic amines *i.e.* creatine / creatinine, sugars and amino acids. The International Agency for Research on Cancer (IARC) has classified red meats and various HCAs on the basis of their mutagenicity/carcinogenicity to humans (IARC, 1993).

Dietary intake of heterocyclic amines even at the levels of ng/kg has become a major risk factor for the aetiology of cancer in humans (Sugimura *et al*., 2004). An obvious increase in global burden of cancer cases has risen up to 18.1 million cases worldwide. Out of the total population (201 million), the incidence of 173,937 new cases of cancers in the already prevailing 310.132 million cancer cases of Pakistan has raised serious health concerns (Bray *et al*., 2018; GLOBOCAN, 2018; IARC, 2018).

The rapid rise in population coupled with escalating urbanization has turned ready-to-eat meat production into a dynamic industry in Pakistan. Keeping in view the prevalent high thermal cooking processes of meat dishes, the health risk concerns of Pakistani community are increasing tremendously. Despite its significance, to the

* Corresponding author: qazi.zool@pu.edu.pk
0030-9923/2020/0006-2397 $ 9.00/0

Abbreviations
HCAs, Heterocyclic amines; RTE, Ready-to-eat; MeIQx, 2-amino-3, 8-dimethylimidazo [4,5-*f*] quinoxaline; IQ, 2-amino-3-methyl-imidazo[4,5-*f*] quinoline; PhIP, 2-amino-1-methyl-6-phenylimidazo [4,5-b] pyridine; ND, Not Detected.

best knowledge of the authors, not even a single study has been conducted yet on it. The objective of present study was to determine the presence of HCAs in RTE chicken kabab samples of different brands commercially available in Pakistan. Influence of various cooking methods, temperatures and durations on the emergence of HCAs in RTE chicken kabab samples is also documented in this communication.

Materials and methods

Three HCAs standards of 2-amino-3-methyl-imidazo [4,5-*f*] quinoline (IQ), 2-amino-3,8-dimethylimidazo [4,5-*f*] quinoxaline (MeIQx) and 2-amino-1-methyl-6-phenylimidazo [4,5-b] pyridine (PhIP) were acquired from Toronto Research Chemicals (Toronto, Canada). Chemicals and solvents such as acetonitrile (HPLC grade), methanol (HPLC grade) ethyl acetate (commercial grade) sodium hydroxide (ACS-grade) diatomaceous earth, millipore filter (25mm, 0.22 micron) and filter paper (47mm, 0.22µm) were purchased from Integrated Biosciences, Karachi-Pakistan.

Commercially available four brands (B, D, S and K) of ready-to-eat (RTE) Chicken Kabab samples were procured from local departmental stores. All samples were divided in two groups. Control group of RTE chicken kabab were processed for extraction after thawing at room temperature. Second group of samples was further cooked by using three high temperature cooking processes recommended by manufacturers of the RTE brands *i.e*. microwave treatment (70-160ºc) pan-frying (150-200ºc) and deep-frying (190-220ºc). Cooking duration, temperatures and respective quantification of HCAs during various cooking methods are shown in Table I.

For Extraction of HCAs from chicken kabab samples, the most commonly used method (Gross and Grueter, 1992) with little modifications (Messner and Murkovic, 2004) was adapted. Accordingly 1g of homogenized kabab sample was taken and mixed well in 12 ml of 1N NaoH. A homogenous suspension was obtained by magnetic stirring of 1h at 600-700 rpm. Mixture was passed through lab-based diatomaceous earth filled column. Twenty milliliter ethyl acetate (C_2H_5COOH) was passed through the Extrelut columns. Cartridges were washed with 2 ml 0.1 N HCL followed by 2 ml MeOH. It was followed by elution of resulting analytes with 2ml MeoH-25% ammonium hydroxide (19:1, ml/ml). Elute was collected.

After Extraction, analysis of HCAs was done by using a High Performance Liquid Chromatography (Perkin Elmer, Total Chrom) with UV/Vis. detector. Separation of HCAs on the basis of their polarity was done by a reversed phase Sharpsil-U C18 Column (250L ×4.6mm, 5µm, 100Aº). Mobile phase A was prepared by the addition of methanol /acetonitrile / water /acetic acid (8ml/14ml/76ml/2ml). pH of the solvent A was adjusted at 5.0 with the help of 25% ammonium hydroxide. Acetonitrile (ACN) was used as solvent B. A linear gradient (100%A, 0-15min; 75%A, 15-30 min.) was used. Injection volume of sample was adjusted at 10µl. Flow rate of mobile phases were adjusted at 0.7ml/min at 258nm.

The limit of detection and limit of quantitation of three HCAs standards IQ, MeIQx and PhIP were found in the range of 0.1-10ng, 0.1-100ng and 10-1000ng, respectively.

Results

The present results showed that the level of heterocyclic amines (HCAs) in the thawed (control) ready-to-eat (RTE) chicken kabab of different brands ranged from 0.97 to 13.66 µg/g. Whereas high temperature cooking procedures, in general, enhanced the levels of HCAs so that they ranged from 1.62 to 22.21 µg/g and 0.71 to 35 µg/g for the different samples following deep frying and microwave cooking, respectively (Table I). Quantitative determination of three HCAs revealed that control (thawed) samples contained high level of PhIP (0.66-12.44 µg/g) followed by MeIQx (nd- 1.22 µg/g) and IQ (nd-0.2 µg/g).

As far as cooking of RTE chicken kabab samples are concerned the results indicated that microwave cooking produced the highest total HCAs content (0.71-35 µg/g) followed by deep-frying (1.62-22.21 µg/g) and pan-frying (1.11-8.57 µg/g). Deep frying was found to produce highest content of both MeIQx and PhIP up to 9.91 µg/g and 10 µg/g respectively. Pan-fried chicken samples were found to contain highest level of IQ (0.25-7.48 µg/g) followed by MeIQx (nd-2.46 µg/g) and PhIP (nd-2.46 µg/g) as shown in Table I.

Percentage quantification of three HCAs (IQ, MeIQx and PhIP) have revealed that highest %-age of PhIP was found for microwave cooking (100%) followed by pan-frying (84%) and deep-frying (50%). Highest %-ages of IQ in microwave, pan and deep-fried samples were found as 100%, 95.05% and 22.4%, in RTE chicken kabab samples cooked for the duration of 5min., 3min. and 2min. respectively. Deep-frying was found to produce highest %-age content of MeIQx up to 77.78% followed by microwave cooking (54%) and pan-frying (42.24%).

IQ has been detected as dominating HCA in microwave treated samples (nd-35 µg/g) followed by pan-fried (0.25-7.48 µg/g) and deep-fried (nd-2.3 µg/g) RTE chicken kabab samples. IQ contents emerged up to 0.25 µg/g, 1.44 µg/g, 4.85 µg/g and 4.97 µg/g at the cooking temperatures of 150-160°c, 160-180°c, 180-190°c and

Table I. Quantitative determination of IQ (2-amino-3-methyl-imidazo[4,5-f] quinolone), MeIQx (2-amino-3,8-dimethylimidazo [4,5-f] quinoxaline) and PhIP (2-amino-1-methyl-6-phenylimidazo [4,5-b] pyridine) and total HCAs (µg/g) in B, D, S and K brands of ready-to-eat chicken kabab cooked for different durations by microwave, pan-frying and deep-frying.

Mode of Cooking	Cooking time (min) / Temperature (°C)	IQ				MeIQx				PhIP				Total HCAs			
		B	D	S	K	B	D	S	K	B	D	S	K	B	D	S	K
Uncooked / Thawed	N.A	ND	0.2	0.08	0.19	1.22	0.4	ND	0.12	12.44	5.03	1.01	0.66	13.66	5.63	1.09	0.97
Microwave	2 / 70	4.8	6.39	2.41	3.9	ND	0.15	1.56	0.15	1.73	1.38	4.39	0.44	6.53	7.92	8.36	4.49
Microwave	3 / 130	4.28	0.84	3.73	35	1.38	0.7	1.81	ND	0.48	0.32	0.76	ND	6.14	1.86	6.3	35
Microwave	4 / 150	ND	3.61	2.00	6.5	1.89	ND	ND	0.62	1.63	2.26	2.00	1.5	3.52	5.87	4.00	8.62
Microwave	5 / 160	0.68	0.4	1.29	ND	0.92	0.44	0.01	ND	1.32	1.27	1.59	0.71	2.92	2.11	2.89	0.71
Deep-Frying	2 / 190-220	2.3	1.4	1.44	ND	9.91	5.74	3.94	0.81	10	0.24	1.06	0.81	22.21	7.38	6.44	1.62
Pan Frying	2 / 150-160	1.55	3.76	5.66	0.25	0.32	0.87	2.46	ND	0.29	1.57	0.36	1.31	2.16	6.2	8.48	1.56
Pan Frying	3 / 160-180	3.1	4.38	0.37	1.44	0.54	2.01	0.05	0.33	0.72	2.18	0.69	0.57	4.36	8.57	1.11	2.34
Pan Frying	4 / 180-190	2.36	0.67	7.48	4.85	0.76	0.49	ND	0.67	2.46	ND	0.9	0.34	5.58	1.16	8.38	5.86
Pan Frying	5 / 190-200	2.04	4.33	4.03	4.97	0.47	0.59	ND	ND	0.52	0.59	0.21	0.31	3.03	5.51	4.24	5.28

N.A, not applied; ND, not detected.

190-200°c, respectively in the pan-fried RTE chicken products. This subsequent rise in the HCAs level with the gradual increase in temperature indicates that production of HCAs can be controlled by lowering cooking temperature.

A detailed quantitative analysis of HCAs in the four commercially available brands (B, D, S and K) indicated that microwave cooking produced highest total HCAs contents in the K brand (35 µg/g) followed by S (8.36 µg/g), D (7.92 µg/g) and B (6.53 µg/g) brands. Deep-frying, however, produced highest total HCAs content in the B brand (22.21 µg/g) followed by D (7.38 µg/g), S (6.44 µg/g) and K (1.62 µg/g) brands. Whereas, pan-frying, produced highest total HCAs content in the D brand (8.57 µg/g) followed by S (8.48 µg/g), K (5.86 µg/g) and B (5.58 µg/g) brands (Table I).

Discussion

The present results agree well to another study, wherein microwave cooking has been found to produce highest HCAs (18.09 ng/g) followed by pan-frying (6.98 ng/g) (Oz *et al*., 2010). The present findings are in conformity to another study which revealed highest detected %-age of PhIP (24%) among HCAs followed by MeIQx (23%) and MeIQ (12%) (Jahurul *et al*., 2010). PhIP contents have been documented in the range of 38.2 ng/g to 46.9 ng/g in the chicken samples cooked by frying (Busquets *et al*., 2004; Keating and Bogen, 2004). Recently, Khan *et al*. (2019) have found highest IQ contents in chicken drumsticks (~ 6.79 ng/g), followed by fried crispy burgers (~4.08 ng/g) and the beef burgers (3.64 ng/g).

Per day estimated dietary consumption of 96, 84, 66 and 63ng of HCAs has been associated with bladder, kidney, colon and rectal cancers, respectively (Augustsson *et al*., 1999). An increased dietary intake of HCAs exceeding 41.4 ng/day could enhance the chances of developing colorectal cancer (Rohrmann *et al*., 2009).

An investigation on dietary median and/or average intake of HCAs/capita/day indicated a relatively higher intake of HCAs up to 1393 ng/day in United States (Keating and Bogen, 2004), followed by Spain (934 ng/day) (Busquets *et al*., 2004) Malaysia (553.7 ng/g) (Jahurul *et al*., 2010) Germany (103 ng/d) (Rohrmann and Becker, 2002) Sweden (77 ng/d) (Augustsson *et al*., 1999) and Singapore (49·95 ng/d) (Wong *et al*., 2005).

Conclusion

The thawed samples were found to harbor HCAs from 0.97 to 13.66 µg/g. After deep-frying and microwave cooking, the RTE chicken kabab samples attained levels of heterocyclic amines (HCAs) ranging from 1.62 to 22.21 µg/g and from 0.71 to 35µg/g, respectively. Conclusively, high thermal processing of the RTE chicken kabab increased their HCAs contents. Keeping this in view, it is speculated that dietary intake of HCAs in this country is highest worldwide (up to 1.4mg/d). A profound link, thereby, can be established between the dietary intake of high thermally processed food and increased incidence of cancer cases in Pakistani population. The higher HCAs contents in RTE chicken Kabab is a point of serious health concern of Pakistani community and draws significant

attention of public health authorities to take necessary measures.

Statement of conflict of interest

The authors declare there is no conflict of interest.

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