DETERMINATION OF HETEROCYCLIC AROMATIC AMINES IN KAVURMA COOKED IN STEAM CAULDRON

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Abstract – This paper describes a method for the determination of nine heterocyclic aromatic amines (HCAs) in commercial kavurma, which were sold in Turkey by high performance liquid chromatography (HPLC) with diode array detector. HCAs are separated on a reversed phase column (Acclaim™ 120, C18, 3µm, 4.6 x 150 mm). **Varying levels 2-amino-3,4-dimethylimidazo[4,5-f]quinoline (MeIQ) (up to 0.13 ng/g), 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx) (up to 21.16 ng/g), 2-amino-3,7,8-trimethylimidazo[4,5-f]quinoxaline (7,8-DiMeIQx) (up to 0.62 ng/g) were detected, while 2-amino-3-methylimidazo[4,5-f]quinoline (IQ), 2-amino-3-methylimidazo[4,5-f] quinoxaline (IQx), 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline (4,8-DiMeIQx), 2-amino-1-methyl-6-phenylimidazo[4,5-b] pyridine (PhIP), 2-amino-9H-pyrido[2,3-b]indole (AαC) and 2-amino-3-methyl-9H-pyrido[2,3-b]indole (MeAαC) were not detected in any of the samples.**

Key Words - High Performance Liquid Chromatography (HPLC), Heterocyclic Aromatic Amines, Kavurma, Solid-Phase Extraction

- INTRODUCTION

Kavurma is a traditional coarsely diced and deep-fried meat product, produced to preserve the meat and has 6–9 months shelf life. In the past, kavurma was utilized only to conserve meat, but currently kavurma is preferred as processed meat. In traditional processing, the beef or mutton is diced (approximately 4x5x6 cm) and mixed with 2–5% salt, and then fried in animal fat using a double-sided steam cauldron. After cooking, it is kept in almost anaerobic conditions by submerging in the solidified animal fat in the container. Recently, it has also become commercially available in vacuum packaged forms produced in modern meat processing plants [1, 2]. Kavurma is considered as a ready-to-eat meat product since it is generally consumed without further processing or cooking (reheating).

HCAs are compounds that formed cooking of proteinaceous foods such as meat [3]. The formation of HCAs in foods is a present concern for food chemists, nutritionists, and toxicologists because of their potential mutagenic/carcinogenic properties [4, 5]. To date, more than 25 different HCAs have been identified in foods, their relative formation is greatly influenced by food composition and cooking methods [4]. To our best knowledge, no previous reports have described on HCAs formation in kavurma, while cooking is main process of its production. Therefore, the aim of the study was to investigate the formation of HCAs in kavurma cooked in steam cauldron.

- MATERIALS AND METHODS

Chemicals

The following chemicals were purchased from Toronto Research Chemicals (Downsview,

Ontario, Canada): IQ (CAS no: 76180-96-6), IQx (CAS no: 108354-47-8), MeIQ (CAS no: 77094-11-2), MeIQx (CASno: 77500-04-0), 4,8-DiMeIQx (CAS no: 95896-78-9), 7,8-DiMeIQx (CAS no: 92180-79-5), PhIP (CAS no: 105650-23-5), AαC (CAS no: 26148-68-5), MeAαC (CAS no: 68006-83-7), 4,7,8-TriMeIQx (CAS no: 132898-07-8, as the internal standard). The stock standard solutions were prepared according to Öz et al. [6]. For the solid phase extraction, an Oasis MCX cartridge (3 cm^3/60 mg, 30 μm) of Waters (Milford, Massachusetts, USA) was used. All the other chemicals were of high performance liquid chromatography (HPLC) or analytical grade. Water was from a Milli-Q water purification system (Millipore, Bedford, Massachusetts, USA). All the solutions were passed through a 0.45-μm filter (Milex, Massachusetts, USA).

Kavurma Samples

Kavurma samples were purchased from local market in Erzurum, Turkey and brought to laboratory under cold chain. The samples were stored -20°C until extraction. They were thawed in refrigerator at 4°C for 12 h and then mixed in a blender to produce a uniform sample prior to extraction. All experiments were repeated three times.

Extraction of Heterocyclic Aromatic Amines

HCAs were extracted from the meat products and purified by using the method described by Messner and Murkovic [7] which is a modified method originally developed by Gross, G.A [8]. According to the method: 1g sample was dissolved in 12 ml 1 M NaOH. The suspension was homogenized by using a magnetic stirring for 1 h at 500 rpm at room temperature. The alkaline solution was mixed with 13 g diatomaceous earth (Extrelut NT packaging material, Merck, Darmstadt, Germany) and then poured into empty Extrelut columns. The extractions were made by using ethyl acetate and the eluate was passed through coupled Oasis MCX cartridges. The cartridge was washed with 2 ml of 0.1 M HCl and 2 ml MeOH. The analytes were eluted with 2 ml MeOH-concentrated (25%) ammonia (19/1, v/v). The eluted mixtures were evaporated to dryness at 50 °C and the final extracts were dissolved in 100 μL MeOH just before measurement.

Identification and Quantification of Heterocyclic Aromatic Amines

Table 2 Data from the quantitative HPLC analysis of the HCAs in kavurma

	IQ	IQx	MeIQx	MeIQ	7,8-DiMeIQx	4,8-DiMeIOx	PhIP	AαC	MeAαC
Kavurma	nd	nd	13.07-21.16	nd-0.13	nd-0.62	nd	nd	nd	nd

HCAs were identified and quantified by HPLC (Thermo Ultimate 3000, Thermo Scientific, USA) with Diode Array (DA) 3000 detector and Ultimate 3000 auto sampler. Separation was carried out on a reversed phase analytical column, Acclaim™ 120 C18 3μm (4.6 x 150 mm) from Tosoh Bioscience GmbH (Stuttgart, Germany) at 30 °C with a mobile phase of methanol/acetonitrile/water/acetic acid (8/14/76/2, v/v/v/v) at pH 5.0 (adjusted with ammonium hydroxide 25%) as solvent A and acetonitrile as solvent B at flow rate 0.7 ml/min. The gradient program was: 0% B, 0-12 min; 0-30% B, 12-20 min; 30% B, 20-25 min. The DAD detection of HCAs was performed at 262 nm and the injection volume was 10μl.

III.RESULTS AND DISCUSSION

Table1 shows the LOD and LOQ values of individual HCAs.

Table1 LOD and LOQ values of HCAs

HCAs	LOD(ng/g)	LOQ(ng/g)
IQx	0,004	0,013
IQ	0,009	0,029
MeIQx	0,024	0,081
MeIQ	0,014	0,047
7,8-DiMeIQx	0,005	0,018

4,8-DiMeIOx	0,008	0,025
PhIP	0,025	0,085
AαC	0,012	0,039
MeαAC	0,01	0,035

The HCA content of Kavurma

This is the first study to describe the levels of HCAs in kavurma in the literature. Data from the quantitative HPLC analysis of the HCAs in kavurma extracted according to the Oasis method, expressed in ng/g kavurma are presented in Table 2. In the present study, IQ, IQx, 4,8-DiMeIOx, PhIP, MeAαC and AαC were not detected in any of samples, but the rest of HCAs were found up to 21.16 ng/g.

IQ was not found in present study, while various IQ levels have been found in the literature. Barnes et al. [9] found 0.53-20.1 ng/g IQ in fried beef at 240 °C for 10 min. Turesky et al. [10] found between 0.3 and 1.9 ng/g IQ in fried beef at 275 °C for 5-15 min. Wakabayashi et al. [11] detected 0.19 ng/g in grilled beef and Felton et al. [12] found from non-detectable to 1 ng/g in fried beef at 200-250 °C for 12 min. IQx was not detected in any of samples in this study, but in various studies, IQx was detected at various levels. Fay et al. [13] found 1.5 ng/g IQx in grilled beef, Turesky et al. [14] also found as 0.12–0.39 ng/g IQx in fried beef at 150-300 °C for 12-24min.

In present study, MeIQx was found between 13.07 and 21.16 ng/g. Rivera et al. [15] was detected 4 ng/g MeIQx in barbecued beef and Turesky et al. [16] found 2.7-12.3 ng/g MeIQx in fried beef at 275 °C for 5-15 min. Felton et al. [12] also detected up to 5.1ng/g MeIQx in fried beef 250 °C for 12 min. However, MeIQx was not detected in commercial cooked beef [16]. MeIQ was detected up to 0.13 ng/g in present study. Similar with this study, Britt et al. [17] found 0.3-0.6 ng/g MeIQ in fried beef at 170 °C for 16 min.On the other hand, Rivera et al. [15] found 8 ng/g MeIQ in barbecued beef at 200-500 °C for 15 min. Knize et al. [18] also found below 0.1ng/g MeIQ in commercially cooked meat, whereas Johansson and Jägerstad [19] did not detect MeIQ in fried beef at 180-190 °C for 12 min.

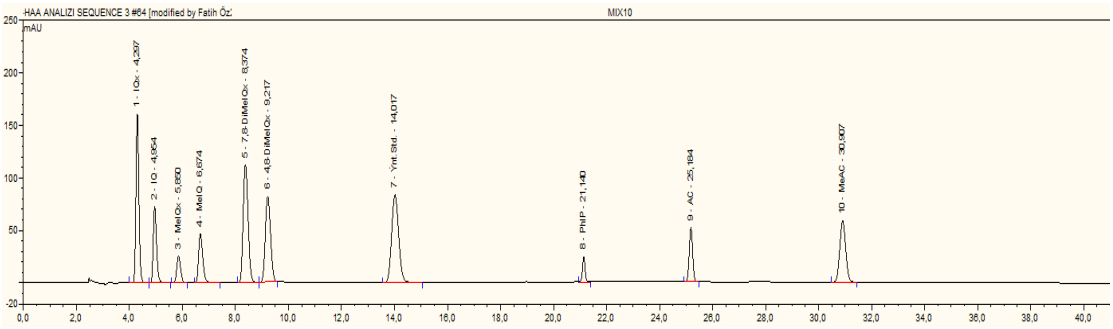
7,8-DiMeIQx was found from non-detectable to 0.62ng/g. Similarly, Turesky et al. [10] found from non-detectable to 0.7ng/g 7,8-DiMeIQx in fried beef at 275 °C for 5-15 min. Fay et al. [13] detected 0.2 ng/g in grilled and Öz et al. [20] found up to 0.28 ng/g 7,8-DiMeIQx in barbecued beef. However, Öz et al. [20] did not detect 7,8-DiMeIQx in fried beef. In present study, 4,8-DiMeIOx was not detected. However, Turesky et al. [10] found up to 3.9 ng/g 4,8-DiMeIOx in fried beef at 275 °C for 5-15 min. Thiébaud et al. [21] also detected 4.5ng/g 4,8-DiMeIOx in fried beef at 277 °C for 12 min. On the contrary, Gross et al. [22] and Öz et al. [20] did not detect 4,8-DiMeIOx in fried beef. PhIP was not identified in present study, but Gross et al. [20] found up to 1.2 ng/g, Thiébaud et al. [21] found 4.5ng/g PhIP in fried beef. Similar with this study, Tikkanen et al. [16] and Öz et al. [20] did not detect PhIP in commercially cooked beef and fried beef.

AαC was not detected in this study. Similar with this study, Felton et al. [12] did not detect AαC in fried beef at 200-250 °C for 12 min. Öz et al. [20] also did not detect AαC in fried beef at 200 °C for 1.5-6min. However, Wakabayashi et al. [11] found 1.2 ng/g, Thiébaud et al. [21] found 21 ng/g AαC in grilled and fried beef. MeAαC was not detected in this study. In other studies, various results have been found. Öz et al. [20] did not detect MeAαC in barbecued and fried beef, while Turesky et al. [14] found up to 0.29 ng/g and 0.14 ng/g MeAαC in barbecued and fried beef, respectively.

IV. CONCLUSION

Many previous studies have shown that cooking methods are very important for the formation

of heterocyclic aromatic amines. The formation of HCAs in kavurma cooked in steam cauldron was investigated in the present study. The results have shown that HCAs could be formed in kavurma. Major HCA was MeIQx in this study and IQ, IQx, 4,8-DiMeIOx, PhIP, AαC and MeAαC were not detected in any of samples.



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