Determination of Heterocyclic Aromatic Amine Content in Turkish Meatball Dishes

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Abstract: In this present study, 2-amino-3-methylimidazo [4,5-f] quinoxaline (IQx), 2-amino-3-methylimidazo [4,5-f] quinoline (IQ), 2-amino-3,8-dimethylimidazo [4,5-f] quinoxaline (MeIQx), 2-amino-3,4-dimethylimidazo [4,5-f] quinoline (MeIQ), 2-amino-3,4,8-trimethylimidazo [4,5-f] quinoxaline (4,8-DiMeIQx), 2-amino-3,7,8-trimethylimidazo [4,5-f] quinoxaline (7,8-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 determined in commonly consumed meatball dishes in Turkey. MeIQ, IQ, MeIQx, PhIP and AαC were the major HCAs and these compounds were found in amounts up to 2.45, 1.91, 1.68, 1.61 and 0.75 ng g^{-1}, respectively. On the other hand, IQx, 7,8-DiMeIQx, 4,8-DiMeIQx and MeAαC were detected at low or under detectable amounts. It was also determined that total HCA amount changed between 0.43 and 6.88 ng g^{-1}. Meatball is commonly consumed meat dish in Turkey and this study provides valuable data that will help estimation of daily HCA intake and exposure.

Key words: Heterocyclic aromatic amines, meatball, solid phase extraction, High Performance Liquid Chromatography (HPLC), Turkey

INTRODUCTION

Several studies reported that diet plays important role in etiology of cancer and one third of human cancers are considered to relate with diet (Sugimura, 2002). Heterocyclic Aromatic Amines (HCAs) are known as mutagenic and/or carcinogenic compounds found ng g^{-1} (ppb) levels in cooked meat and fish (Murkovic, 2004). Until now, >25 HCAs have been isolated and identified from cooked foods (Puangsombat et al., 2012). After evaluations of long-term animal studies in vitro and in vivo genotoxicity tests, The International Agency for Research on Cancer (IARC) regarded HCAs as possible human carcinogens (MeIQ, MeIQx and PhIP, class 2B) and as probable human carcinogens (IQ, class 2A) (IARC, 1993). Especially IQ and MeIQ were referred to as super mutagens (Busquets et al., 2004). The carcinogenicity of HCAs are also reported dozen times higher than those of aflatoxin, amine nitrite and benzo(a)pyrene (Dong et al., 2013).

HCAs can be classified into two main groups called IQ-type (Thermic HCAs) and non IQ-type HCAs (Pyrolitic HCAs). IQ-type HCAs are formed by heat induced non-enzymatic browning known as Maillard reaction which involves creati(ni)ne, amino acid and sugar whereas pyrolitic HCAs are mainly formed by pyrolysis of amino acids and proteins at higher temperatures above 300°C (Busquets et al., 2004; Sanz Alaejos et al., 2008). It is reported that various factors such as cooking conditions, type of meat, fat, moisture, pH, sugar, free amino acid, creati(ni)ne contents of meat, lipid oxidation and antioxidants are the main parameters that influence variety and amount of HCAs (Oz and Kaya, 2011).

Many epidemiological studies, investigated the association between well-done meat intake and cancer risk have shown that high well-done meat intake and high exposure to HCAs may increase the risk of human cancer (Zheng and Lee, 2009). In this respect, investigating applications and habits increase or decrease human cancer risk and determination of HCAs in cooked foods are important for evaluation of nutrition and cancer interaction. Meatball is commonly consumed meat dish in Turkey and even in world. Therefore, quantification of HCA content in most consumed meat dishes such as meatballs can help estimation of daily HCAs intake and exposure. The aim of this study was to determine the HCA content of commonly consumed meatball dishes that can be major contribution of daily HCA intake as well as exposure.

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MATERIALS AND METHODS

Chemicals: All chemicals and solvents that used for HCA analysis were of HPLC or analytical grade. Water was obtained from Diamond NANOpure water purification system (Barnstead, Dubuque, Iowa, USA). Chemicals for HCA analysis were acetone (Merck, Germany), acetonitrile (Merck, Germany), ethyl acetate (Ridel-de Haen, France), methanol (Sigma Aldrich, Germany), sodium hydroxide (Sigma Aldrich, Czech Republic), hydrochloric acid (Sigma Aldrich, Germany), ammonium hydroxide solution (25%, Sigma Aldrich, Germany), glacial acetic acid (Sigma Aldrich, Germany). In addition for solid phase extraction Extrelut NT packing material (Merck, Germany), Oasis MCX cartridges (Waters, USA 3 cm^3/60 mg) were used. All solutions were passed through a 0.45 μm filter (Milex, Massachusetts, USA) before use. HCA standards were purchased from Toronto Research Chemicals (Downswiev, Ontario, Canada). Stock standard solutions were prepared according to Oz *et al.* (2007).

Meatball samples: Commonly consumed 9 meatball dishes were investigated. Samples were obtained without dressing and condiments from mass catering institutions in Ankara, Turkey. In this study, sampling was created according to frequencies of meatball dishes in the menu and their preferability. Standard recipes and standard basis weight of ingredients for each meatball dish were recorded. Table 1 shows ingredients of each meatball dish. Cooking temperature of each sample was measured with surface thermometer (Testo905-T2, Lenzkirch, Germany) and internal temperature of each sample was measured with food thermometer (Testo905-T1, Lenzkirch, Germany) by inserted temperature probe horizontally to the midpoint of the sample. Cooking time was also measured with chronometer. Uncooked and cooked samples were weighted and cooking loss was calculated as a percentage after cooked samples were allowed to cool at room temperature for approximately 30 min. Samples were homogenized by kitchen blender (Tefal, France) and wrapped by aluminum foil and stored at -20°C until HCA extraction and were thawed in a refrigerator at 4°C for 12 h prior to extraction.

Cooking process: Cooking procedures of each meatball dish are given in Table 2. As can be seen in Table 2, baking and grilling were major cooking methods and in present study seven dishes were baked. Cooking temperatures, internal temperatures and cooking times of the samples ranged between 150 and 200°C, 73.4 and 82.1°C, 6.4 and 81 min, respectively. Mean cooking losses also ranged between 15.80 and 18.58, 14.05 and 28.80% for grilled and baked samples, respectively. According to safe cooking temperature chart published by US Food and Drug Administration (FDA, 2013) minimum internal temperature of beef patties should be 71.1°C for microbiological safety. Thus, it was determined that all meatball samples were microbiologically safe.

Moisture, total fat and pH analysis: Moisture content, total fat and the pH of samples were analyzed for both uncooked and cooked meatball samples. Moisture content was analyzed by using infrared moisture analyzer (Sartorius MA 150, Goettingen, Germany) according to

Table 1: The ingredients of meatball dishes

Meatball dishes	Cooking method	Ingredients
Dish A	Grilling	Ground beef, onion, breadcrumbs, parsley, red pepper, black pepper, cumin, salt, garlic and sunflower oil
Dish B	Grilling	Ground beef, onion, breadcrumbs, parsley, red pepper, black pepper, cumin, salt, garlic, sunflower oil, egg and allspice
Dish C	Baking	Ground beef, onion, salt, black pepper, meatball spices and sunflower oil
Dish D	Baking	Ground beef, salt, egg, onion, red pepper, cumin, red pepper, breadcrumbs, garlic and sunflower oil
Dish E	Baking	Ground beef, salt, egg, onion, black pepper, cumin, red pepper, garlic, breadcrumbs and sunflower oil
Dish F	Baking	Ground beef, salt, egg, onion, black pepper, cumin, red pepper, garlic, breadcrumbs and sunflower oil
Dish G	Baking	Ground beef, egg, parsley and sunflower oil
Dish H	Baking	Ground beef, onion, garlic, breadcrumbs, egg and sunflower oil
Dish I	Baking	Ground beef, onion, parsley and breadcrumbs

Table 2: Cooking procedures of meatball dishes

Meatball dishes	Cooking temperature (°C)	Internal temperature (°C)	Cooking time (min)	Uncooked Moisture (%)	Total fat (%)	pH	Cooked Moisture (%)	Total fat (%)	pH	Cooking loss (%)
Dish A	184±15.9	80.1±4.8	6.4±2.10	54.36±3.60	16.66±4.70	5.84±0.05	55.45±1.60	12.65±0.73	6.00±0.08	18.58±8.650
Dish B	185±7.1	80.5±1.8	8.0±0.10	54.88±5.83	10.26±0.82	5.74±0.04	50.52±6.97	15.70±5.56	5.89±0.04	15.80±13.63
Dish C	164±21.9	80.0±4.5	81.0±20.1	55.79±2.17	12.27±4.88	5.81±0.11	56.67±1.38	10.83±2.49	6.04±0.09	22.13±0.810
Dish D	150±0.1	74.8±0.2	73.3±28.9	55.26±2.55	13.13±7.25	5.79±0.11	60.26±4.92	7.74±3.21	5.98±0.09	28.80±3.240
Dish E	168±20.5	79.4±5.5	32.0±16.4	58.40±7.31	11.35±6.90	5.73±0.12	58.54±7.43	9.46±3.99	5.97±0.15	19.98±8.830
Dish F	170±0.1	81.6±2.2	30.0±0.10	61.40±2.02	7.80±0.72	5.71±0.04	57.38±5.39	10.24±3.11	5.95±0.11	26.07±0.690
Dish G	165±7.1	78.0±2.8	42.5±3.50	59.38±2.71	10.71±5.42	5.75±0.10	59.86±2.20	8.15±0.21	5.93±0.10	17.82±5.230
Dish H	200±0.1	82.1±0.1	30.0±0.10	58.67±3.31	14.85±4.01	5.77±0.11	56.03±2.21	10.97±0.47	5.95±0.05	16.86±3.370
Dish I	150±0.1	73.4±1.6	22.0±4.50	61.28±4.72	10.71±3.20	5.73±0.02	51.71±5.68	14.53±3.13	5.94±0.04	14.05±2.390

Data was given as mean±standard deviation

manufacturer's instruction. Total fat and the pH of samples were determined according to Vural and Oztan (1996). Total fat was measured by Soxhlet Method extracting with petroleum ether. For the pH measurement, 10 g of sample was weighted and added 100 mL of distilled water. The mixture was homogenized with Ultra-Turrax T 25 basic (IKA, Staufen, Germany) for 30 sec at medium speed, after that the pH of samples was measured using pH meter (EDT instruments GP 353, Dover, UK) which calibrated with pH 4 and 7 solutions.

Extraction of heterocyclic aromatic amines: HCAs were extracted from the samples and purified by using the method described by Messner and Murkovic (2004) which is a modified method originally developed by Gross and Gruter (1992). This method (Oasis Extraction Method) has some advantages compared with original method. This method allowed clean up all HCAs in only one fraction and in one step. According to this method, 1 g of cooked sample was dissolved in 12 mL 1 M NaOH and the suspension was homogenized by using magnetic stirrer at 500 rpm at room temperature for 1 h. The alkaline solution was mixed with 13 g Extrelut NT packing material and then poured into empty Extrelut columns. The extractions were made by washing with ethyl acetate and elute was passed through the coupled Oasis MCX cartridges by vacuum system. The cartridges were washed with 0.1 M HCl (2 mL) and MeOH (2 mL). The analytes were eluted with 2 mL MeOH-concentrated (25%) ammonia (19 L^{-1}, v/v). The eluted mixtures swere evaporated to dryness at 50°C and the final extracts were dissolved in 100 μL internal standard (4,7,8-TriMeIQx, 10 ng g^{-1}) in methanol just before HCA analysis.

Identification and quantification of heterocyclic aromatic amines: HCAs were identified and quantified by HPLC (Thermo Separation Product Spectra System P1000, Thermo Scientific, USA) with ultraviolet (UV) 3000 detector and AS 3000 auto sampler. Separation was carried out on a reversed phase analytical column, Semi Micro ODS-80 TS column (5 μm, 250 mm×2 mm i.d) 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 0.3 mL/min. The gradient program was: 0% B, 0-12 min; 0-30% B, 12-20 min; 30% B, 20-25 min. The UV detection of HCAs was performed at 262 nm and the injection volume was 5 μL. For quantification of HCAs, internal standard addition method was used.

Analytical conditions and recoveries: As analytical quality insurance, the identities of analyte peaks were established by comparing retention times of analyte peaks with standard HCA solutions and spiked samples. In this regard, calibration curves were used for quantifying HCAs. Coefficient of regression (R^2) for individual HCA in mix solution was calculated by performing linear regression analysis (nanogram of each compound against peak area). Determined R^2 values were 0.9999 for Iqx, 1.0000 for IQ, 0.9996 for MeIQx, 0.9999 for MeIQ, 1.0000 for 7,8-DiMeIQx, 0.9996 for 4,8-DiMeIQx, 0.9997 for 4,7,8-TriMeIQx, 0.9994 for PhIP, 0.9997 for AαC, 0.9996 for MeAαC. Each peak area of individual HCA was expressed as ng g^{-1} of cooked sample.

Recovery rate for each analyzed HCA in sample was determined by Standard Addition Method. The samples were spiked at four spiking levels (0.5, 1.0, 2.5 and 5 ng g^{-1} freezed sample) by adding different volumes of a methanolic solution of analytes. The determined recoveries depend on the sample nature and spiked concentration level. The average recoveries of the HCAs were 78% for Iqx, 65% for IQ, 76% for MeIQx, 79% for MeIQ, 73% for 7,8-DiMeIQx, 78% for 4,8-DiMeIQx, 98% for PhIP, 62% for AαC, 68% for MeAαC. These results are comparable to those in literature (Polak *et al.*, 2009; Oz, 2011; Puangsombat *et al.*, 2012).

The Limit of Detection (LOD) and Limit of Quantification (LOQ) for standard solutions were calculated with a signal to noise ratio of 3 (S/N = 3) and 10 (S/N = 10), respectively. The lowest detection levels were 0.003 ng g^{-1} for IQx, 0.002 ng g^{-1} for IQ, 0.006 ng g^{-1} for MeIQx, 0.005 ng g^{-1} for MeIQ, 0.003 ng g^{-1} for 7,8-DiMeIQx, 0.005 ng g^{-1} for 4, 8-DiMeIQx, 0.005 ng g^{-1} for PhIP, 0.008 ng g^{-1} for AαC, 0.008 ng g^{-1} for MeAαC. The lowest quantified levels were 0.01 ng g^{-1} for IQx, 0.01 ng g^{-1} for IQ, 0.02 ng g^{-1} for MeIQx, 0.02 ng g^{-1} for MeIQ, 0.01 ng g^{-1} for 7,8-DiMeIQx, 0.02 ng g^{-1} for 4,8-DiMeIQx, 0.02 ng g^{-1} for PhIP, 0.03 ng g^{-1} for AαC, 0.03 ng g^{-1} for MeAαC. Figure 1 shows HPLC chromatogram of mix standard solution (10 ng g^{-1}).

Statistical analyses: In the present study, the experimental design was completely randomized design with two replicates. All data statistically analyzed by using SPSS 15.0 statistical package (SPSS, Inc., Chicago, IL). p<0.05 was considered statistically significant.
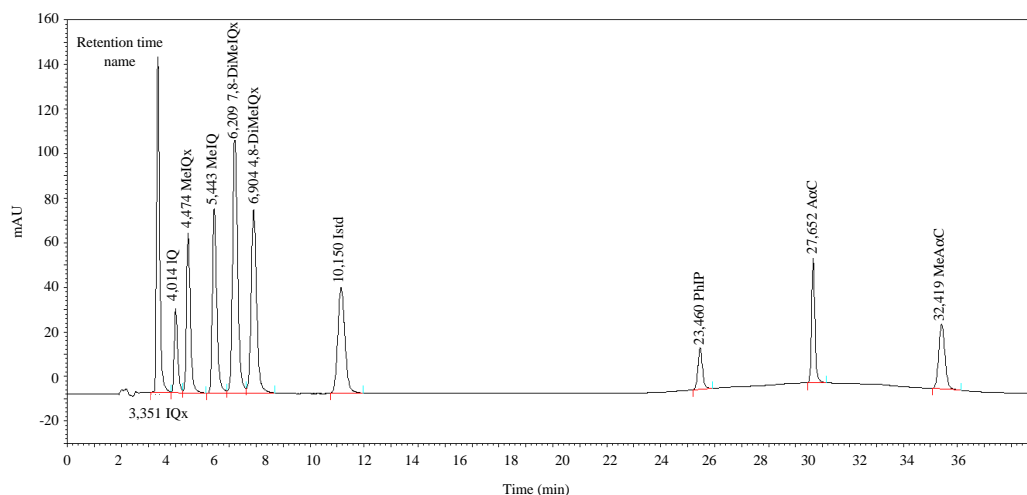
Fig. 1: HPLC chromatogram of a solution of nine different HCA standards (10 ng g^{-1}). 4,7,8-TriMeIQx is internal standard (10 ng g^{-1})

Table 3: HCA contents of meatball dishes (ng g^{-1})

Meatball dishes	IQx	IQ	MeIQx	MeIQ	7.8-DiMeIQx	4.8-DiMeIQx	PhIP	AαC	MeAαC	Total HCAs
Dish A	0.08	1.91	0.16	0.12	0.09	0.05	0.70	0.57	ND	3.68
Dish B	0.01	0.36	0.06	0.10	ND	ND	0.48	0.14	ND	1.15
Dish C	0.39	0.87	1.18	2.45	0.05	0.15	1.04	0.75	ND	6.88
Dish D	0.04	0.55	1.68	ND	0.06	ND	1.61	0.29	0.61	4.84
Dish E	ND	1.09	0.29	0.51	ND	ND	1.26	0.34	ND	3.49
Dish F	ND	ND	ND	ND	ND	ND	ND	0.43	ND	0.43
Dish G	ND	0.32	ND	0.34	0.26	ND	0.51	0.21	ND	1.64
Dish H	0.11	0.36	0.06	0.24	0.06	ND	1.24	0.14	ND	2.21
Dish I	ND	1.13	0.80	ND	ND	ND	1.01	ND	ND	2.94

Data was given as mean; ND = Not Detected

RESULTS AND DISCUSSION

Moisture, total fat and pH analysis: The mean moisture content, total fat and the pH of samples are given in Table 2. Moisture content, total fat and the pH of uncooked samples were determined to range from 54.36-61.40, 7.80-16.66, 5.71-5.84%, respectively. On the other hand, moisture content, total fat and the pH of cooked samples were determined to range from 50.52-60.26, 7.74-15.70, 5.89-6.04%, respectively. In the literature, similar results have been found for cooked meatball samples. Oz (2011) found moisture and total lipid contents of ready to eat meatballs at the rate of 50.78-55.11, 13.24-17.61%, respectively, sold in restaurants in Turkey.

Heterocyclic aromatic amine contents of samples: Meatballs are commonly consumed meat dishes in Turkey and even in world. Thus, determination of the HCA contents in commonly consumed meat dishes is important for estimation of daily HCA intake or exposure. This study describes carcinogenic and/or mutagenic HCA levels of Turkish meatball dishes and in literature; it is first study

in terms of determination of HCA content in nine mostly consumed meatball dishes. Besides these results are important issue for consumers. In the present study varying levels of HCAs were detected. The highest detected HCA was MeIQ (2.45 ng g^{-1}) followed by IQ (1.91 ng g^{-1}), MeIQx (1.68 ng g^{-1}), PhIP (1.61 ng g^{-1}) and AAC (0.75 ng g^{-1}). However, IQx, 4,8-DiMeIQx, 7,8-DiMeIQx were detected trace amount and MeAαC was only detected in one dish sample (dish D). Table 3 shows mean HCAs levels of each meatball dish.

In literature, generally identified HCAs are reported as PhIP, MeIQx, 4,8-DiMeIQx, IQ and MeIQ in cooked meats whereas it was reported that other HCAs detected less frequently (Balogh *et al.*, 2000; Knize *et al.*, 1995). However, in present study, 4,8-DiMeIQx was detected low or non-detectable level. This result is in agreement with other studies that investigate HCA levels of commercially cooked meats (Tikkanen *et al.*, 1993; Wong *et al.*, 2005).

IQ was one of the most abundant HCA in all meatball dishes. It was detected to range from non-detectable to 1.91 ng g^{-1}. In the literature several researchers found similar IQ levels in ground beef patties and hamburgers.

Oz (2011) found 0.94 ng g^{-1} in ready to eat meatballs. Oz and Kizil (2012) detected 1.95 ng g^{-1} in cooked commercial frozen meat products. In another study, IQ level of cooked hamburgers was found 0.58 ng g^{-1} (Klassen *et al.*, 2002). In this study, 1.91 ng g^{-1} IQ was found in grilled samples (dish A) cooked at 184°C for 6.4 min. Similarly, 1.7 ng g^{-1} IQ was found in fried ground beef patties cooked at 200°C for 6 min per side (Balogh *et al.*, 2000).

MeIQ was another major HCA in this study and detected up to 2.45 ng g^{-1}. However, in three baked dishes (dish D, F and I) MeIQ were not detected. In addition except dish C, MeIQ contents of samples were generally found at low or trace amounts in both grilled and baked meatball dishes. The highest MeIQ level was detected in dish C. In addition, AαC and total HCAs content of dish D were also the highest. It has been recently showed that cooking time and temperature have most significant effect on HCA formation. Particularly this significant effect reported for MeIQ and PhIP contents (Dundar *et al.*, 2012). The mean cooking time of dish D is 81 min therefore, the highest level of MeIQ, AαC and total HCAs can be explained by longer cooking time. In other studies that analyze HCA levels of commercial meatballs or hamburgers, MeIQ were detected between below 0.1 and 0.66 ng g^{-1} (Klassen *et al.*, 2002; Oz, 2011). However, in fried hamburgers MeIQ was not quantified (Busquets *et al.*, 2004).

MeIQx and PhIP have been generally reported as major HCAs in cooked meat products (Salmon *et al.*, 2006). However, in present study MeIQx was found at trace levels except three baked meatball dishes (dish C, D and I) that have 0.80 ng g^{-1} or higher MeIQx levels. In literature, some researchers found similar results. Polak *et al.* (2009) detected 0.15 ng g^{-1} and Murray *et al.* (1993) found 0.5 ng g^{-1} MeIQx in grilled beef samples. MeIQx was also found 0.83 ng g^{-1} in cooked commercial frozen meat products (Oz and Kizil, 2012).

PhIP was another most abundant HCA for all meatball dishes and detected to range from non-detectable to 1.61 ng g^{-1}. It was observed that PhIP levels of baked meatballs (expect dish F and G) were slightly higher than grilled ones but these differences were not statistically significant (p>0.05). In other studies, PhIP levels were varied. Warzecha *et al.* (2004) detected 1.9 ng g^{-1} in pan fried beef while Oz *et al.* (2010) did not detect in grilled meat at 200°C for 2-8 min. On the other hand, PhIP was found 0.1-0.6 ng g^{-1} (Knize *et al.*, 1995) and 1.38 ng g^{-1} in commercial hamburgers (Klassen *et al.*, 2002). In addition, Oz (2011) detected 1.19 ng g^{-1} PhIP in ready to eat meatballs.

IQx, 7,8-DiMeIQx and 4,8-DiMeIQx were found generally at trace or non-detectable amount in all meatball dishes. There were limited studies that investigate IQx level in literature. Turesky *et al.* (2005) found at the levels ranging from 0.03-0.20 ng g^{-1} in barbecued beef. However, Oz *et al.* (2010) detected between at non-detectable level and 3.65 ng g^{-1} in barbecued beef whereas same researchers did not detect IQx in grilled beef samples. In this study, IQx level was found between at non-detectable level and 0.39 ng g^{-1} but Oz (2011) found at the levels ranging from 1.59-3.81 ng g^{-1} in ready to eat meatballs.

As can be seen in Table 3, 7, 8-DiMeIQx and 4,8-DiMeIQx were detected at levels up to 0.26 and 0.15 ng g^{-1}, respectively. In the literature, Busquets *et al.* (2004) found similar results. They detected <0.04 ng g^{-1} 7,8-DiMeIQx and <0.1 ng g^{-1} 4,8-DiMeIQx in fried beef hamburgers. In another study, 4,8-DiMeIQx and 7,8-DiMeIQx were not detected or found at trace amount in commercially cooked beef (Wong *et al.*, 2005). In addition, Tikkanen *et al.* (1993) did not detect 4,8-DiMeIQx while Toribio *et al.* (2007) detected at levels ranging from 0.28-0.72 ng g^{-1} in commercially cooked beef samples.

In present study, AαC was detected up to 0.75 ng g^{-1} whereas MeAαC was detected only one sample (dish D, 0.61 ng g^{-1}). Similarly, Toribio *et al.* (2007) found 0.33 ng g^{-1} AαC in griddled beef cooked at 180-210°C for 4 min. Busquets *et al.* (2004) also detected 0.5 ng g^{-1} AαC and 0.4 ng g^{-1} MeAαC in griddled beef steak. Pyrolitic HCAs are mainly formed by pyrolysis of amino acids and proteins at higher temperatures above 300°C (Sanz Alaejos *et al.*, 2008). Therefore, detection of pyrolitic HCAs at non-detectable level or low amount was expected result in this study when considered cooking procedures of the samples.

Total HCAs levels of meatball samples were detected between 0.43 and 6.88 ng g^{-1}. In various studies similar results were found. Total HCAs concentrations were found 5.54 ng g^{-1} in commercially cooked meatballs (Oz, 2011), 3.61 ng g^{-1} in commercial hamburgers (Klassen *et al.*, 2002) and up to 3.1 ng g^{-1} in commercially cooked beef (Zimmerli *et al.*, 2001).

The studies on quantification of HCA concentration in cooked meat and meat products have reported conflicting results in literature because of the differences of meat types, origin of meat, ingredients that use to prepare the meatball, cooking procedures, analyzing methods, number of HCAs that analyzed. Therefore, comparing the results with other studies is difficult.

CONCLUSION

This first study that investigated HCA levels of commonly consumed Turkish meatball dishes obtained from mass catering can be used as a databank in further researches that will estimate HCA intake and exposure. Meatball dishes have varying HCA levels. IQ, MeIQ,

MeIQx, PhIP and AαC were found major HCAs in this study. However, MeIQx and MeIQ were slightly higher in baked dishes than grilled ones. Further studies will be focus on chicken, beef and fish dishes in order to complete HCA databank of meat dishes in Turkey.

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