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Emergence of mutagenic/carcinogenic heterocyclic amines in traditional Saudi chicken dishes prepared from local restaurants

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ABSTRACT

In the current investigation, five most potential HAs (MeIQx, 4,8-DiMeIQx, IQ, MeIQ and PhIP) were analyzed in traditional Saudi chicken dishes (shawaya, Ala Al-Faham, kebab, saleeg, mandi, kabsa and madhbi) prepared from local restaurants. The aims of the present study were to identify the presence of HAs in cooked chicken dishes, and to conclude how the levels and types of HAs could be affected by cooking methods and food ingredients. In control samples, HAs were found at higher levels ranged from not quantified to 33.72 ng/g. Nonetheless, in chicken dishes, the HAs (MeIQx, 4,8-DiMeIQx and PhIP) amounts are varied at higher range and relatively detected at lower levels from not quantified to 16.35 ng/g, IQ and MeIQ were not identified in any of the studied chicken dishes except shawaya where found to be not quantified. The HAs reduction rates were obtained at higher values in all of the studied samples, among them mandi sample demonstrates the reduction rates higher than 70%, whereas saleeg sample shows the reduction rates almost 100% except PhIP (~95%). The obtained outcomes have markedly showed that HAs occurrence in thermally processed chicken dishes is extremely affected from both cooking methods and addition of food ingredients.

1. Introduction

Heterocyclic amines (HAs) are possibly carcinogenic chemical substances produced in thermally processed proteinaceous foods for instance meat and fish (Barzegar et al., 2019; Khan et al., 2009a,2013; Khan, 2015a). The formation of HAs occurs when meat and/or fish amino acids and creatine or creatinine react at common cooking conditions (Gibis, 2016; Puangsombat et al., 2012). The HAs amounts were increase when the meat products thermally treated for longer time or meat turned blackened, until now, more than twenty five HAs have been identified as a result of meat cooking (Barzegar et al., 2019; Gibis, 2016). In research laboratory investigations, HAs have been detected to be mutagenic, they can cause alterations in deoxyribonucleic acid after metabolizing with particular enzymes present in the human body, possibly will rise the threat of cancer disease allied with the exposure to these chemicals (Agudo et al., 2009; Nöthlings et al., 2009; Cai et al., 2016; Cross and Sinha, 2004; Bellamri et al., 2018). Investigations on experimental animals have revealed that HAs exposure to such animals can produce cancer of the liver, colon, breast, lungs, mammary and prostate (Shirai et al., 1997; Malik et al., 2018). Based on such evidences, the International Agency for Research on Cancer (IARC) in the World Health Organization has listed seven types of HAs (a) MeIQ, MeIQx, PhIP, Glu-P-1, Glu-P-2, AαC, and MeAαC as *possible carcinogens to humans* (Group 2B) and (b) IQ as *probable carcinogen to humans* (Group 2A) (International Agency for, 1993). Recently, the US National Toxicology Program has also registered a number of HAs (MeIQx, IQ, MeIQ and PhIP) as "*reasonably anticipated human carcinogens*" (National Toxicology Progr, 2004). The IARC has assessed the carcinogenicity of the eating of red and processed meat, listed the consumption of red and processed meat as "*probably carcinogenic to humans*" and "*carcinogenic to humans*", respectively (Domingo and Nadal, 2017). Besides, the investigators have recognized several constituents which exhibited immensely great mutagenic effectiveness through the *Ames test* 100 to 100000 times superior than other compounds for instance polycyclic aromatic hydrocarbons and *N*-nitroso compounds (De Stefani et al., 1997; Takayama et al., 2008).

Lately, Khan et al. have described the HAs identification in home cooked camel dishes and restaurant prepared burgers particularly of those eaten by Saudi Arabian population (Khan, Mu and Alothman, 2017; Khan et al., 2015b). In previous studies, various restaurant foods

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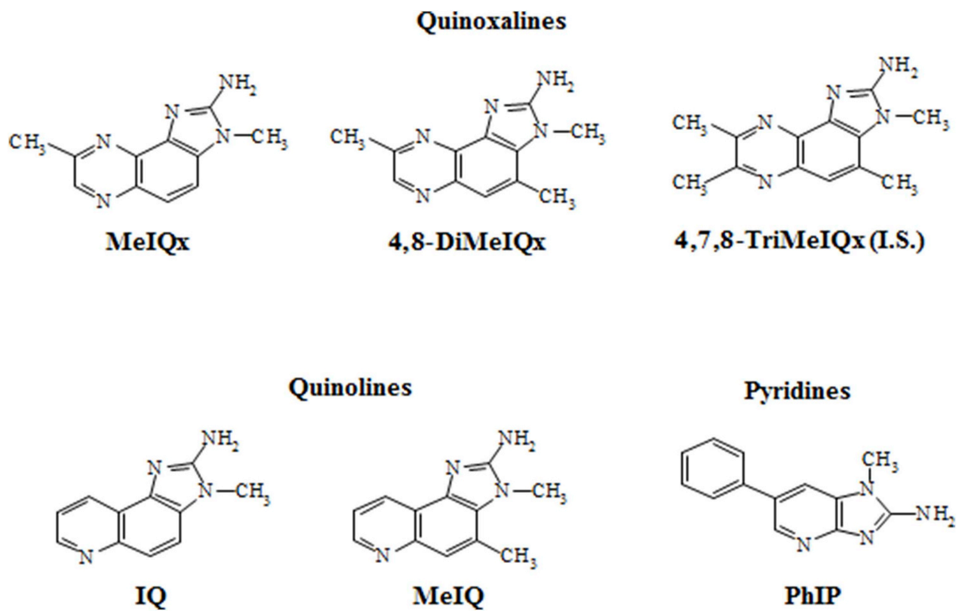

Fig. 1. HAs structures and abbreviated names studied in cooked chicken samples.

have been examined for HAs levels (Khan et al., 2009a, Khan et al., 2019a; Sabally et al., 2016; Öz and Zikirov, 2015; Salmon et al., 2006; Knize et al., 1998). Despite the fact, the evidence resulting from such investigation assists as an indispensable base of information from a public health perspective, it also can offer indications to considerate the influences that affect HAs occurrence and can specify means of decreasing or eradicating these carcinogenic chemicals.

Chicken is one of the highest frequently consumed foods worldwide, among them the Saudis are regarded as the world's major consumers of chicken (http://english.alarabiya.net/en/business/markets/2016/03/14/Saudis-among-world-s-largest-consumers-of-chicken.html) and, it is expected that the production of chicken meat in Saudi Arabia will be reached up to 700000 million tons in the year 2019 (Poultry and Products Annu, 1811). Some common Saudi Arabian dishes are ubiquitous in the whole Kingdom, among them the chicken dishes are very popular especially prepared at local restaurants, and could be a substantial source of HAs exposure.

Consequently, the investigation of the correlation concerning HAs and their role in the attribution of the human cancer cause necessities the accurate identification of HAs in chicken meat especially prepared at restaurants. The objectives of the current study were to identify the presence of HAs in cooked chicken dishes obtained from local restaurants, to make public new prospective causes of HAs, and to conclude how the levels and types of HAs could be affected by the cooking methods and addition of food ingredients naturally applied during cooking of meat products. Nonetheless, the cooking methods and the use of food ingredients are thought to be a favorable measure to reduce HAs levels owing to their capability as obstructs HAs occurrence or as quashing agents on HAs metabolic rate (Khan, 2015a; Vitaglione and Fogliano, 2004; Khan et al., 2016; Khan et al., 2019b).

2. Materials and methods

2.1. Chemicals and materials

Solvents for instance ethyl acetate, acetonitrile and methanol (LC grade, ≥99.9%) were obtained from Sigma-Aldrich (St. Louis, USA). Ammonium acetate and sodium hydroxide of reagent grade (≥98%), and acetic acid of ReagentPlus® (≥99%) were purchased from Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany). Ammonia solution (25%) was obtained from Supelco (Darmstadt, Germany). The HAs (MeIQx, 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline, 4,8-DiMeIQx, 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline, PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine, IQ, 2-amino-3-methylimidazo[4,5-f]quinoline, and MeIQ, 2-amino-3,4-dimethylimidazo[4,5-f]quinolone) were purchased from Toronto Research Chemicals (Toronto, Canada, purity > 99%). The structures and abbreviated names of studied HAs have been displayed in Fig. 1.

HAs standard stock solutions at concentration 100 µg/g were prepared in water and methanol (50:50, v/v), solutions were applied for additional dilution purposes. The HAs solutions were refrigerated (+4 °C) until instrumental analysis. The HAs standard mixtures including internal standard (I.S., 4,7,8-TriMeIQx, 0.5 µg/g) have been prepared by mass to form the linearity range and calibration curves in all methods. The samples and HAs solutions were filtered by means of PTFE syringe filter of 0.22 µm pore size (Macherey-Nagel GmbH, Düren, Germany) before being analysis using ultra-performance liquid chromatography-tandem mass spectrometry (UPLC) system. Sample preparation was carried out using ultrapure Milli–Q water obtained from Millipore purification system (Bedford, USA).

For solid-phase extraction (SPE) method, the extraction cartridges (Bond Elut propylsulfonyl silica (PRS, 500 mg) and octadecylsilane C_{18} (100 mg) including stoppers and connecters were purchased from Varian (Harbor City, USA). The Extrelut NT20 extraction column were obtained by Merck (Darmstadt, Germany), and diatomaceous earth (refill material) was purchased from Agilent Technologies (Apple Valley, USA).

To ground the meat samples, a blender (Microtron MB 550, Kinematica AG, Littau, Switzerland) was used. Homogenization of the ground meat samples with sodium hydroxide (1 M) solution were performed using an Ultra-Turrax® T25 basic (IKA, Staufen, Germany) grinder. Sample extraction and pre-concentration were carried out using vacuum manifolds (Visiprep™ and Visidry™), obtained from Supelco (Gland, Switzerland). Samples were kept in a glass vials containing screw cap having PTFE seal (Thermo Scientific, Rockwood, USA).

2.2. Sample processing

Seven individual chicken meat (control samples, without addition of food ingredients) and chicken dishes (with addition of food ingredients) for instance shawaya, Ala Al-Faham, kebab, saleeg, mandi, kabsa and

Table 1
Details of the food processing methods of traditional Saudi recipes.

Sample	Food ingredients	Cooking method	Uncooked chicken (g)	Cooking time (min)	Cooked meat (g)	Weight loss (%)
Shawaya[a]	Whole chicken (1000 g), olive oil (5 mL)	Pan-frying	1000	10	504	49.6
Shawaya	Whole chicken (1000 g), lemon juice (10 mL), hot paprika (10 g), chat masala (5 g), yogurt (5 mL), fennel seed (5 g), black pepper corns (5 g), chopped shallots (3 units, 100 g), sliced garlic (5 cloves, 50 g), vinegar (5 mL), olive oil (5 mL) and salt (5 g)	Pan-frying	1000	10	563	43.7
Ala Al-Faham[a]	Whole chicken (1000 g)	Grilled (charcoal)	1000	25	648	35.2
Ala Al-Faham	Whole chicken (1000 g), onion (1 unit, 40 g), chopped ginger (20 g), garlic (2 cloves, 5 g), coriander (20 g), lemon juice (5 mL), olive oil (5 mL), Arabic masala (2.5 g), pepper (10 g) and salt (5 g).	Grilled (charcoal)	1000	25	712	28.8
Kebab[a]	Boneless chicken (1000 g)	Grilled (charcoal)	1000	20	665	33.5
Kebab	Boneless chicken (1000 g), yogurt (230 g), olive oil (10 mL), paprika (10 g), cumin (3 g), parsley (1 g), crushed red pepper flakes (5 g), peeled tomato (30 g), black pepper powder (3 g), minced garlic (5 cloves, 25 g), sliced onion (1 unit, 50 g), and salt (5 g)	Grilled (charcoal)	1000	20	744	25.6
Saleeg[a]	Whole chicken (1000 g)	Boiling and Broiling (oven)	1000	30	681	31.9
Saleeg	Whole chicken (1000 g), hot water (1000 mL), rice (200 g), cardamom (2 units, 10 g), cloves (2 unit, 5 g), milk (100 mL) and salt (5 g)	Boiling and Broiling (oven)	1000	30	754	24.6
Mandi[a]	Chopped whole chicken (1000 g)	Broiling (oven)	1000	35	677	32.3
Mandi	Chopped whole chicken (1000 g), rice (400 g), onion powder (2 unit, 80 g), minced garlic (5 g), cloves 25 g, lemon juice (20 mL), black pepper (2.5 g), chili peppers (2.5 g), cardamom (5 unit, 20 g), cloves (5 unit, 20 g), cinnamon sticks (3 unit, 20 g), saffron (5 g) and salt (5 g)	Broiling (oven)	1000	35	748	25.2
Kabsa[a]	Chicken (800 g)	Broiling (oven)	800	30	563	29.6
Kabsa	Chicken (800 g), rice (300 g), chopped onion (1 unit, 30 g), chopped tomato (30 g), tomato paste (15 mL), raisins (64 g), vegetable oil (30 mL), baharat spice (10 g), ground cardamom (15 g), chopped garlic (4 unit, 20 g), dried black lime (10 g), cinnamon stick (1 unit, 5 g), salt (5 g)	Broiling (oven)	800	30	618	22.7
Madhbi[a]	Whole chicken (1000 g)	Baking	1000	30	622	37.8
Madhbi	Whole chicken (1000 g), olive oil (20 mL), turmeric (0.1 g), baharat spice (10 g), chopped onion (0.2 g), cardamom (5 unit, 20 g), cloves (5 unit, 20 g), saffron (5 g) and salt (5 g)	Baking	1000	30	708	29.2

[a] Control samples, cooked without food ingredients.

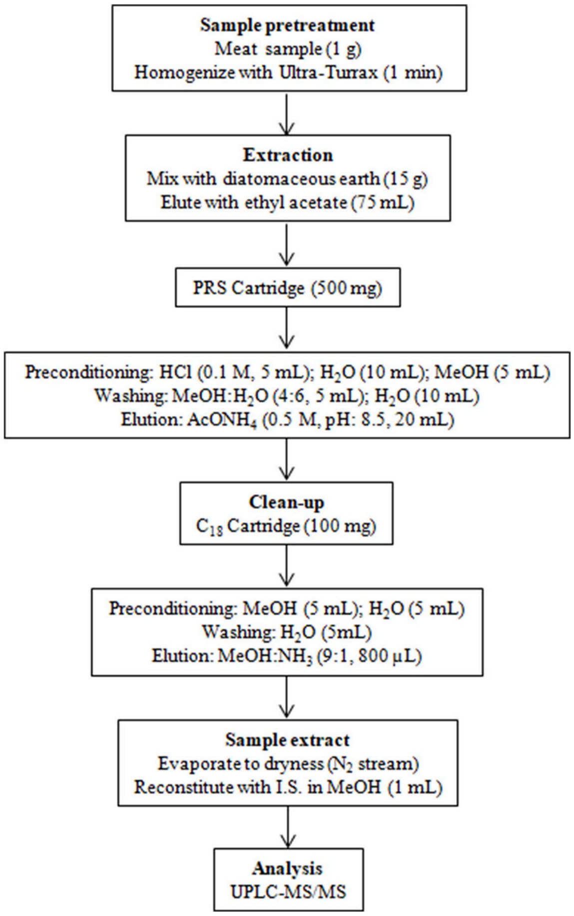

Fig. 2. The HAs extraction using solid-phase extraction method.

Table 2
MRM conditions used with the triple quadrupole instrument.[a]

HAs	Precursor ion $[M+H]^+$, *m/z*	Quantification		Confirmation	
		Product ion, *m/z*	Collision energy (eV)	Product ion, *m/z*	Collision energy (eV)
MeIQx	214	199	30	131	25
4,8-DiMeIQx	228	213	30	187	25
4,7,8-TriMeIQx[b]	242	227	25	201	30
IQ	199	184	30	157	35
MeIQ	213	198	25	197	30
PhIP	225	210	25	183	30

[a] Dwell time (0.025 s) in each analyte.
[b] Internal standard.

madhbi were purchased from the local restaurants in Riyadh, Saudi Arabia. About the preparation of control samples, it has been requested to the respective restaurants to prepare in the similar way as chicken dishes were prepared. The chicken dishes were chosen on the basis of relatively most commonly eaten dishes compared to other meat dishes, information was obtained from various local restaurants and individuals in Riyadh, Saudi Arabia. The meat quantity, food ingredients, cooking methods, cooking time, and weight loss have been demonstrated in Table 1. Before sampling, the visible food ingredients, skin and bones were removed. The weight loss was estimated as the difference between sample weight before and after the thermal treatment. Finally, the samples were pulverized, sieved, bottled, tagged and stored at a very low temperature (−20 °C) until analysis.

2.3. HAs extraction and purification

The extraction and purification of HAs from thermally processed chicken meat samples were carried out using previously developed technique (Gross and Grüter, 1992) and slightly amended by Toribio and co-workers (Toribio et al., 2007). Initially, the refrigerated chicken meat samples were taken out and allowed to equilibrate at room temperature. Meat sample approximately 15 g was weigh up in falcon tube and added nearly 30 g of sodium hydroxide (1 M). The sample solution was homogenized using blender (Ultra-Turrax® T25). Subsequently, a mass equal to 1 g of meat was carefully mixed with dispersing material (diatomaceous earth, 13 g). An empty Extrelut column (60 mL) combined to a Bond Elut PRS cartridge (500 mg) was filled with sample, previously the PRS cartridge was preconditioned with 5 mL hydrochloric acid (0.1 M), 10 mL water and 5 mL methanol. The targeted HAs were removed from diatomaceous earth followed by elution to the PRS cartridge using 75 mL of ethyl acetate. Once the elution completed, the PRS cartridge was dried under vacuum followed by washing with a mixture of water and methanol (60:40, 15 mL) and 2 mL water. After that, the Bond Elut C_{18} cartridge was conditioned with 15 mL methanol and 5 mL water, and linked with PRS cartridge. The HAs was eluted from PRS cartridge to C_{18} cartridge by means of 0.5 M ammonium acetate (20 mL) at pH 8.5. Finally, C_{18} cartridge was washed using 5 mL water and dried under vacuum followed by HAs elution with a mixture solution of ammonia/methanol (1:9, v/v, 800 μL). The sample was dried under nitrogen gas of high purity, the dried extracts were redissolved with 100 μL of 4,7,8–TriMeIQx (internal standard, 0.5 μg/g). The sample solution was filtered using PTFE syringe filter of 0.22 μm pore size, and collected into UPLC vials. Each samples were analyzed in three replicates (n = 3). The extraction procedure has been displayed in Fig. 2.

Standard addition method was applied to calculate the HAs levels including recovery values. The standard addition method comprised two zero levels (unspiked) and three spiked levels (50%, 100% and 300%). The calculation of recovery values were obtained from the slope of the linear regression, achieved between the spiked HAs quantity and the calculated HAs quantity. The statistical analysis was performed by ANOVA method.

2.4. HAs separation and identification

An UPLC model Waters Acquity® was used to separate the HAs, coupled with a quaternary pump (Milford, USA). Analytical column based on reversed phase BEH C_{18} (Waters Acquity®) with dimension 50 mm × 2.1 mm id and 1.7 mm particle size was applied. Binary mobile phase comprise organic solvent acetonitrile (A) and 30 mM formic acid/ammonium formate buffer at pH 4.75 (B), and the flow (1 mL/min) were used for the separation of HAs. The mobile phase at gradient elution profile was, 5% A (0–0.1 min); 5–30% A (0.1–1.5 min); 30–60% A (1.5–1.8 min); 60% A (1.8–2.4 min). The sample injection volume (10 μL) was used (Barcelo-Barrachina et al., 2004). For precise analysis and to evade column contamination, the column was washed using water and methanol mixture (50:50, v/v) for 5 min after every ten sample analysis.

For precise HAs identification, a triple quadrupole mass spectrometer (MS/MS) model Micromass Quattro Premier (Milford, USA) was applied, coupled with electrospray ionization (ESI) source. The positive ionization and multiple reaction monitoring (MRM) modes were applied with mass spectrometer system. The ESI source working parameters were as follows: capillary voltage, 3.5 kV; cone voltage, 42 V; desolvation temperature, 350 °C; source temperature, 120 °C; desolvation gas flow rate, 800 L/h; cone gas flow rate, 80 L/h. Nitrogen as cone gas was produced from nitrogen generator (NM30LA, Peak Scientific, Inchinnan, United Kingdom), and the argon as a collision gas was used. A rotary pump oerlikon, model SOGEVACSV40 BI (Cedex, France) was used to provide the MS vacuum. The MRM parameters applied with the

Table 3
Levels of HAs in Saudi traditional chicken dishes prepared with various cooking methods and food ingredients.

Sample	MeIQx (ng/g ± sd**)	4,8-DiMeIQx (ng/g ± sd)	IQ (ng/g ± sd)	MeIQ (ng/g ± sd)	PhIP (ng/g ± sd)
Shawaya[a]	3.14 ± 0.03	1.92 ± 0.02	0.22 ± 0.10	0.15 ± 0.10	33.72 ± 0.50
Shawaya	2.75 ± 0.02	1.63 ± 0.01	nq	nq	16.35 ± 0.40
Ala Al-Faham[a]	1.89 ± 0.02	1.54 ± 0.02	nq	nq	12.65 ± 0.30
Ala Al-Faham	1.56 ± 0.02	1.12 ± 0.02	nd	nd	8.52 ± 0.20
Kebab[a]	2.31 ± 0.02	1.52 ± 0.02	nq	nq	10.95 ± 0.30
Kebab	1.84 ± 0.01	1.04 ± 0.01	nd	nd	7.35 ± 0.20
Saleeg[a]	nq	nq	nd	nd	2.60 ± 0.02
Saleeg	nq	nq	nd	nd	1.85 ± 0.02
Mandi[a]	0.89 ± 0.01	0.36 ± 0.01	nd	nd	7.20 ± 0.20
Mandi	0.63 ± 0.01	0.20 ± 0.01	nd	nd	5.68 ± 0.10
Kabsa[a]	0.90 ± 0.01	0.34 ± 0.01	nd	nd	1.18 ± 0.01
Kabsa	0.76 ± 0.01	0.25 ± 0.01	nd	nd	0.77 ± 0.02
Madhbi[a]	2.88 ± 0.03	1.55 ± 0.02	nd	nd	15.97 ± 0.30
Madhbi	2.29 ± 0.01	1.10 ± 0.01	nd	nd	12.52 ± 0.30

[a] Control samples (cooked without food ingredients); **standard deviation (standard addition calibration, n = 3); nq, not quantified; nd, not detected.

mass analyzers have been illustrated in Table 2. The instrumental data acquisition was carried out by the Waters MassLynx V4.1 software (Milford, USA) (Barcelo-Barrachina et al., 2004).

3. Results and discussion

The five highly potential HAs MeIQx, 4,8-DiMeIQx, IQ, MeIQ and PhIP have been analyzed in most traditional Saudi chicken dishes (shawaya, Ala Al-Faham, kebab, saleeg, mandi, kabsa and madhbi) prepared from local restaurants. The obtained outcomes from the investigated samples are illustrated in Table 3. Results showed that among five HAs only three of them (MeIQx, 4,8-DiMeIQx and PhIP) were most commonly identified in both control samples (except shawaya) and chicken dishes. In control samples, MeIQx, 4,8-DiMeIQx and PhIP were found at higher levels ranged from not quantified to 33.72 ng/g. Nonetheless, in chicken dishes, MeIQx, 4,8-DiMeIQx and PhIP amounts were varied at higher range and relatively detected at lower levels from not detected to 16.35 ng/g, In shawaya control sample, IQ and MeIQ were also identified at lower concentrations 0.22 ng/g and 0.15 ng/g, respectively. The values were found in good agreement with the values found in earlier investigations (Khan et al., 2009; Solyakov and Skog, 2002), where applied identical cooking method including temperature monitoring in meat samples. Chicken dishes, usually prepared by different cooking methods and food ingredients, and relatively produce lower amounts MeIQx, 4,8-DiMeIQx and PhIP, however IQ and MeIQ were not identified in any of the studied chicken dishes except shawaya where found to be not quantified. The results obtained from the investigated samples illustrate that the HAs concentrations decrease using different cooking methods and food ingredients (Khan, 2015a; Vitaglione and Fogliano, 2004; Khan et al., 2016; Khan et al., 2019b; Liao et al., 2010).

The recovery values and limit of detection (LOD, signal-to-noise, 3:1) of the analyzed samples have been demonstrated in Table 4. Recovery values (58–73%) and LOD (0.008–0.014 ng/g) were obtained in control samples. However, in chicken dishes, recovery (50–68%) and LOD (0.012–0.042 ng/g) were obtained. The higher recovery and LOD have been detected in control samples compare to the chicken dishes, these causes might be due to the presence of food ingredients which intrude the extraction performance of samples. In previous literature, the authors have described that the HAs recovery values mostly varies from 5 to 98% because of the intricacy of the sample matrices and involvement of many SPE processes (Toribio et al., 2000, 2007). These values were found in good agreements with those values obtained in previous studies (Jinap et al., 2018; Toribio et al., 2000, 2007). These agreements can be due to the application of same extraction and detection techniques and meat products (Jinap et al., 2018). To demonstrate the outcomes, the HAs chromatograms obtained in madhbi sample have been shown in Fig. 3. The great sensitivity was attained while performing the analysis, however various MRM transitions were carried out at one time.

HAs MeIQx, 4,8-DiMeIQx, IQ, MeIQ and PhIP were detected in control samples, among them IQ and MeIQ were found at lower concentrations. For the formation of these amines usually elevated food preparation temperature is needed (Ahn and. Grün, 2005). Conversely, HAs MeIQx and 4,8-DiMeIQx are frequently formed at higher levels at food preparation temperatures ranged between 200 °C and 210 °C (Khan et al., 2009). Regarding PhIP, usually the formation occurs at higher concentration in white meats for instance cooked chicken which was found in the equivalent value to the amount obtained in previously investigated thermally processed chicken meat (Khan et al., 2009). Nonetheless, the formation of PhIP occurred at lower concentration in cooked red meats for instance in cooked beef, camel and offal (Khan et al., 2009, 2019b; Khan, 2015a). Recently, Khan and coworkers have also determined the highest amount of PhIP (121 ng/g) in another proteinaceous cooked food (swordfish) (Khan et al., 2013). This is the highest amount of PhIP has been determined so far, and is higher than those frequently found in cooked beef, chicken, chicken and offal products (Khan et al., 2009a, Khan et al., 2009b, 2019b; Khan, 2015a). On the basis of previous studies, PhIP has been typically illustrated as one of the utmost adulterated meat products, and supposed to be as one of the primary exposure to the ingestion of HAs in our day-to-day living existence (Busquets et al., 2013).

3.1. Effect of cooking conditions

The influence of cooking conditions and food ingredients on the formation of HAs in cooked chicken dishes have also been recognized, outcomes displayed in Table 3. Comparatively, the HAs amounts in thermally processed chicken dishes were identified at lower HAs amounts than the control samples. The high reduction rates of HAs were observed in all analyzed chicken dishes, some samples for instance mandi has resulted the reduction rates higher than 70%, whereas saleeg has resulted the reduction rates almost 100% except PhIP (~95%). The amounts of HAs obtained using these cooking methods are in the same range with the earlier investigations (Busquets et al., 2004; Skog et al., 2003; Domingo, 2011; Öz et al., 2010). In grilled chicken (Ala Al-Faham and kebab) no IQ and MeIQ have been indentified, whereas MeIQx (1.56–1.84 ng/g), 4,8-DiMeIQx (1.12–1.04 ng/g) and PhIP (8.52–7.35 ng/g) were obtained, these values were found similar IQ (not detected) or slightly lower MeIQ (< 0.1 ng/g), MeIQx (0.3 ng/g), 4,8-DiMeIQx (0.4 ng/g) and PhIP (2.3 ng/g) than those obtained in previous study (Busquets et al., 2004). These varirations may be due to the application of longer cooking time (20–25 min) than in earlier study (13 min) (Busquets et al., 2004). It was find out that the boiling chicken meat comprised no MeIQx, 4,8-DiMeIQx and PhIP (Solyakov and Skog, 2002), except PhIP (1.85 ng/g), the values for MeIQx and 4,8-DiMeIQx are found similar to those obtained in the present study. Öz et al. (2010) has also affirmed that chicken sample cooked using various cooking methods (oven, microwave, barbequing, hot plate and pan-frying) contained HAs in only barbecued samples (Öz et al., 2010). Broiling of samples (saleeg, mandi and kabsa) produced lower amounts of MeIQx and 4,8-DiMeIQx, and PhIP up to 5.68 ng/g, the IQ and MeIQ were not identified in any of these samples. These values were found in good agreement with most literature data where applied similar cooking method (Solyakov and Skog, 2002). In Ala Al-Faham and kebab, the cooking method grilled (charcoal) was applied where MeIQx, 4,8-DiMeIQx, and PhIP were detected from 1.04 to 8.52 ng/g, these values are slightly higher than those samples cooked with broiling method. However, the IQ and MeIQ were also not identified in these samples. The archived values are found to be similar attained in previous study (Solyakov and Skog, 2002). Madhbi was cooked by baking method, where MeIQx and 4,8-DiMeIQx were detected at concentration 2.29 ng/g and 1.10 ng/g, respectively, PhIP was indetified at concentration 12.52 ng/g, the IQ and MeIQ were not detected. These values were found similar to those samples cooked by broiling methods in the present study and also to those achieved in earlier literature (Solyakov and Skog, 2002). Shawaya was cooked by pan-frying method, the MeIQx and 4,8-DiMeIQx, and PhIP were detected at higher concentractions (1.63–16.35 ng/g) than those achieved in other dishes, and more close to the values obtained in control sample and also in previous literatue (Khan et al., 2009b). The reason may be due to the application of similar cooking conditions.

3.2. Effect of food ingredients

The use of food ingredients play an important role on the formation of HAs and have capability to reduce their generation while cooking of meat and fish products. Despite the fact, evidence resulting from such investigation assists as an indispensable awareness as of a community health perspective, it also can make available indications to considerate the reasons that affect HAs occurrence and can designate the way of decreasing or removing such types of carcinogens. Numerous investigations have observed other features that may influence the formation of HAs, for instance addition of food ingredients, vitamins and marinate before or with cooking process (Khan, 2015a; Khan et al., 2016; Khan et al., 2019b; Vitaglione et al., 2002). The samples cooked with food ingredients (Table 1) have revealed a great reduction on HAs generation (Table 3). Such protective influences have been recognized because of the presence of different antioxidant constituents such as flavonoids and polyphenolics compounds (Vitaglione et al., 2002). Onion, ginger and garlic are the major food ingredients used in cooked foods, and known to be the key sources of dietary flavonoids and have high antioxidant properties (Haskaraca et al., 2014, 2017; Johansson and Jägerstad, 1996; Murkovic et al., 1998; Stoilova et al., 2007; Nuutila et al., 2003). A number of studies have also shown that the garlic and onion application can result to enhance the concentrations of some HAs, on the whole it causes a reduction in the total HAs amount (Nuray and Öz, 2019). In the current study, many dishes contain these food ingredients and showed the HAs reduction at higher levels (Table 3). In another study, the authors have illustrated that the use of tomato as food ingredient also diminish the formation of HAs nearly 70% in both chemical and meat juice model systems (Öz et al., 2010). The HAs values in current investigation were found in good agreement with the HAs values attained in the earlier study (Öz et al., 2010). Nevertheless, in shawaya the reduction was not attained at higher rates, this low reduction probably because of the properties of radical scavenging in various food ingredients which may have hinder the HAs formation (Nuray and Öz, 2019). As a result, the chicken meat prepared by means of food ingredients show the competence to reduce the formation of HAs. These data could be explicated because of the use of

Table 4
Assessed HAs recovery (R), limits of detection (LOD) and limit of quantification (LOQ) in studied samples.

Sample	MeIQx			4,8-DiMeIQx			IQ			MeIQ			PhIP		
	R, (%)	LOD, ng/g	LOQ, ng/g	R, (%)	LOD, ng/g	LOQ, ng/g	R, (%)	LOD, ng/g	LOQ, ng/g	R, (%)	LOD, ng/g	LOQ, ng/g	R, (%)	LOD, ng/g	LOQ, ng/g
Shawaya*	63	0.012	0.037	65	0.013	0.040	70	0.012	0.037	64	0.012	0.037	68	0.013	0.040
Shawaya	50	0.041	0.124	53	0.034	0.103	53	0.033	0.100	50	0.042	0.127	52	0.033	0.100
Ala Al-Faham*	58	0.013	0.040	60	0.014	0.043	65	0.010	0.031	63	0.012	0.037	65	0.010	0.031
Ala Al-Faham	53	0.033	0.100	51	0.031	0.094	59	0.024	0.073	55	0.032	0.097	57	0.022	0.067
Kebab*	60	0.012	0.037	63	0.010	0.031	67	0.013	0.040	60	0.013	0.040	64	0.010	0.031
Kebab	53	0.031	0.094	56	0.023	0.070	54	0.032	0.097	51	0.041	0.124	55	0.021	0.064
Saleeg*	62	0.010	0.031	65	0.010	0.031	68	0.011	0.034	69	0.010	0.031	68	0.010	0.031
Saleeg	55	0.013	0.040	57	0.014	0.043	60	0.021	0.064	63	0.021	0.064	63	0.012	0.037
Mandi*	65	0.008	0.025	65	0.008	0.025	73	0.009	0.031	66	0.009	0.031	69	0.008	0.025
Mandi	60	0.012	0.037	56	0.013	0.040	68	0.013	0.040	61	0.013	0.040	62	0.011	0.034
Kabsa*	58	0.012	0.037	60	0.013	0.040	64	0.013	0.040	60	0.011	0.034	60	0.014	0.043
Kabsa	51	0.034	0.103	53	0.032	0.097	57	0.032	0.097	52	0.023	0.070	54	0.021	0.064
Madhbi*	61	0.012	0.037	63	0.012	0.037	62	0.011	0.034	58	0.014	0.043	59	0.013	0.040
Madhbi	52	0.033	0.100	55	0.033	0.100	54	0.032	0.097	50	0.042	0.127	53	0.032	0.097

LOD = signal-to-noise ratio of 3:1; LOQ = signal-to-noise ratio of 10:1; *Control samples (cooked without ingredients).

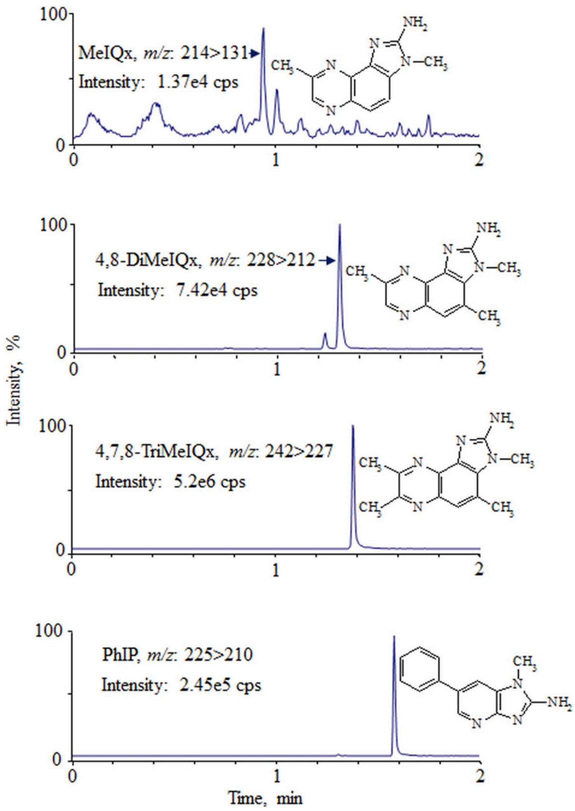

Fig. 3. Liquid chromatography–tandem mass spectrometry chromatograms of HAs detected in madhbi sample.

various food ingredients which have the antioxidant properties, and may have pro-oxidative influences with the successive peroxyl radicals formation or through the foraging of free radicals or oxygen (Vitaglione and Fogliano, 2004; Khan et al., 2019b; Johansson and Jägerstad, 1996; Gibis and Weiss, 2012). Nonetheless, the use of cooking methods in the current investigation are not so simple to evaluate with the earlier investigation because of more than a few researchers have used a combination of many food ingredients along with various cooking methods (Lan et al., 2004). Likewise, some investigators have also defined the use of simply one type of ingredient and a number of cooking methods for HAs attenuation in meat samples (Persson et al., 2003). In order to access the HAs exposure in our daily life, it is highly essential to scrutinize the meat and use of food ingredients type, and cooking methods including cooking time and temperature in epidemiological investigations.

4. Conclusions

In the current investigation, the five highly potential HAs (MeIQx, 4,8-DiMeIQx, IQ, MeIQ and PhIP) were examined in most traditional Saudi chicken dishes (shawaya, Ala Al-Faham, kebab, saleeg, mandi, kabsa and madhbi). To our knowledge, this is the first investigation about the occurrence of HAs in Saudi chicken dishes especially prepared from local restaurants. The obtained outcomes have markedly showed that HAs occurrence in thermally processed chicken dishes is extremely affected from both food ingredients and cooking methods. The HAs (MeIQx, 4,8-DiMeIQx and PhIP) amounts are varied at higher range and relatively detected at lower amounts between not quantified to 16.35 ng/g sample, however the HAs IQ and MeIQ were not identified in any of the studied chicken dishes except shawaya where found to be not quantified. The high HAs reduction rates were obtained in all of the studied samples, in some samples for instance mandi where reduction rates were observed more than 70%, and in saleeg the reduction rates were found to be nearly 100% except PhIP (~95%). The outcomes from the investigated samples showed that the HAs amounts decrease using different cooking methods comprising low direct heat contact with meat and food ingredients contain antioxidants which have pro-oxidative influences with the successive occurrence of peroxyl radicals or due to the scavenging of oxygen or free radicals. In order to assess the human HAs consumption, the outcomes from the current investigation could be applied at global level and offers to find the best cooking methods and food ingredients that reduce the threat of HAs exposure, and therefore to promote the safety and quality of the food products especially protein-rich foods that generate HAs carcinogens after cooking process.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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