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EFFECT OF NATURAL FOOD CONDIMENTS ON CARCINOGENIC/MUTAGENIC HETEROCYCLIC AMINES FORMATION IN THERMALLY PROCESSED CAMEL MEAT

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ABSTRACT

In the present study, the effect of various natural food condiments (garlic, ginger, pepper, tomato and onion) on the formation of heterocyclic amines (HAs) in cooked camel meat was studied. In control sample, the amount of HAs MeIQx, 4,8-DiMeIQx and PhIP were obtained between 2.10 and 5.22 ng/g, while, MeIQ and IQ were found less than quantification limit. The camel meat cooked with different food condiments, the HAs were found in lesser amounts. MeIQx, 4,8-DiMeIQx and PhIP were detected from 0.42 to 2.83 ng/g, however, MeIQ and IQ were not indentified in any analyzed samples. Consequently, camel meat cooked by various food condiments illustrates the capability to reduce the HAs formation. Such information may be elucidated because of the presence of antioxidants in such condiments which might have pro-oxidative effects with the subsequent formation of peroxyl radicals or by the scavenging of oxygen or free radicals.

PRACTICAL APPLICATIONS

This is the first study relating to the inhibition of HAs by means of some common food condiments in cooked camel meat. In this work, five HAs such as MeIQx, 4,8-DiMeIQx, PhIP, MeIQ and IQ have been studied in camel meat samples thermally processed with or without food condiments. Our results evidently demonstrate that the formation of HAs in cooked camel meat is highly affected by using food condiments. The current study also illustrates that the concentrations of HAs can be kept at low levels by cooking the meat that include food condiments. The obtained results could be used to estimate the human intake of HAs either in Saudi Arabia or global and supplied to the search of good food condiments that reduce the threat of exposure to HAs, and thus to advance the food security and quality.

INTRODUCTION

Heterocyclic amines (HAs) are known to be as mutagenic/carcinogenic substances occurred at very low concentrations during the cooking of protein-rich food (pyrolysis of amino acids, creatine and proteins) for instance meat and fish (Schut and Snyderwine 1999; Nagao and Sugimura 2000; Sugimura *et al.* 2004). In the aetiology of human cancer, the HAs have also been discussed, moreover, many epidemiological investigations have revealed a relationship between a high consumption of meat and a greater threat of rising cancers (Sugimura 1992, 2000; Alaejos *et al.* 2008; World Cancer Research Fund/American Institute for Cancer Research 2009). Lately, a number of animal investigations have exposed that HAs are influential carcinogens which induce tumors in a range of organs such as pancreas (Anderson *et al.* 2002), colon (Nowell *et al.* 2002), stomach and esophagus (Ward *et al.* 1997).

So far, over 25 various HAs have been recognized in protein-rich cooked foods such as meat and fish (Skog and Solyakov 2002; Borgen and Skog 2004; Busquets *et al.* 2004;

Salmon *et al.* 2006; Khan *et al.* 2008, 2009a, 2009b; Jahurul *et al.* 2010; Puangsombat *et al.* 2012; Khan *et al.* 2013, 2015a;).

The major food condiments for instance creatine, amino acids and sugar are involved in the generation of HAs cooking toxicant (Messner and Murkovic 2004). The amounts and types of HAs generated can be ascribed to the conditions such as cooking time, temperature and procedures in addition to the composition of raw protein-rich foodstuffs (Busquets *et al.* 2004; Khan *et al.* 2009a,b; Busquets *et al.* 2013; Khan *et al.* 2013, 2015a). Based on adequate evidences relating to the genotoxicity and carcinogenicity in experimental animals. The National Toxicology Program (NTP), 13th *Report on Carcinogens*, has classified MeIQx, PhIP, IQ, MeIQ as *reasonably anticipated to be a human carcinogen* (NTP 2014). The International Agency for Research on Cancer (IARC) has also listed IQ as *probable human carcinogen*, while, MeIQx, PhIP and MeIQ as *possible human carcinogens* (IARC 1993). Consequently, to investigate the association between HAs carcinogen and cancer, and biomarkers of revelation in biological samples require to be determined (Busquets *et al.* 2013).

Camel meat is a very popular and frequently eaten in the Arab countries especially in the Saudi Arabia. It is usually thermally treated by different cooking methods including a variety of food condiments were added together or at intermission period. In many literatures, it has been illustrated that the addition of food condiments which has antioxidant properties, diminishes the formation of HAs (Balogh *et al.* 2000; Vitaglione and Fogliano 2004; Gibis and Weiss 2012). To diminish the HA formation, antioxidants show a potential measure because of their capability as inhibitors of HA generation or as suppressing/inhibiting agents on HA metabolism/biotransformation (Vitaglione and Fogliano 2004).

The objective of the present work was to study for the first time the effect of various food condiments (garlic, ginger, pepper, tomato and onion) on the formation of HAs during frying of camel meat. Moreover, the current investigation seeks to determine foods with little quantity of HAs, so as to be able to recommend meat that could be employed to prepare derived healthier meat products. Previous literature illustrates the screening of HAs levels in cooked camel meat (Khan *et al.* 2015a), thus it was essential to study the effect of food condiments on the formation of HAs in such foods.

MATERIALS AND METHODS

Chemicals and Materials

Acetonitrile, methanol and ethyl acetate of HPLC grade were purchased from Merck (Darmstadt, Germany). Ammonium formate, ammonium acetate and formic acid (98%) were supplied from Merck (Darmstad, Germany). Ammonia solution (25%) and sodium hydroxide were obtained from Panreac Quimica (Barcelona, Spain) and BDH Laboratory Supplies (Poole, UK), respectively. The chemicals were of analytical grade. Milli-Q water purification system, Advantage A10 (Millipore Corporation, Bedford, USA) was used to purify the water.

Bond Elut propylsulfonyl silica PRS (500 mg), octadecylsilane C_{18} (100 mg) cartridges, coupling pieces, stopcocks were supplied from Varian (Harbor City, USA). Extrelut NT20 extraction cartridges were obtained from Merck (Darmstad, Germany). Inert diatomaceous earth hydromatrix bulk material was purchased from Agilent Technologies (Apple Valley, USA). Amber flasks (40 mL) with PTFE seal and screw cap (Thermo Scientific, Rockwood, USA) were used to store the samples.

HAs, 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx), 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-3-methylimidazo[4,5-f]quinoline (IQ), 2-amino-3,4-dimethylimidazo[4,5-f]quinoline (MeIQ) and 2-amino-3,4,7,8-tetramethyl-imidazo [4,5-f]quinoxaline (4,7,8-TriMeIQx) (Fig. 1), were purchased from Toronto Research Chemicals (Toronto, Canada). The purity of HAs was higher than 99% and 4,7,8-TriMeIQx was used as an internal standard (I.S.). Individual stock standard solutions of HAs at concentration of 100 μg/g were prepared in methanol and used for further dilutions. To set up the range of linearity and for the building of calibration curves, standard mixtures solution of HAs (MeIQx, 4,8-DiMeIQx, PhIP, IQ and MeIQ) at concentrations range from 0.1 to 1.0 μg/g containing 4,7,8-TriMeIQx (0.5 μg/g, I.S.) were prepared by weight. Before injection into the ultra-performance liquid chromatography (UPLC) system, the samples and solutions were filtered through a 0.45 μm PTFE filter (MACHEREY-NAGEL GmbH & Co., Düren, Germany).

For solvent evaporation and SPE technique, Visidry™ and Visiprep™ vacuum manifolds (Supelco, Gland, Switzerland) were used. The type-K insulated-wire probes, monitored with Normadics TC6 software (Cole-Parmer, Vernon Hills, USA) was used to measure the cooking temperature of camel meat samples. The Microtron® MB 800 (Kinematica AG, Littau, Switzerland) was used to blend the cooked meat samples.

Preparation of Food Samples

To our knowledge, this is the first study relating to the formation and inhibition of most potential five HAs in six types of camel meat dishes which have been pan-fried with or without using food condiments. Firstly, the camel meat sample was cooked without applying any food condiments and considered as a control sample. Subsequently, the camel

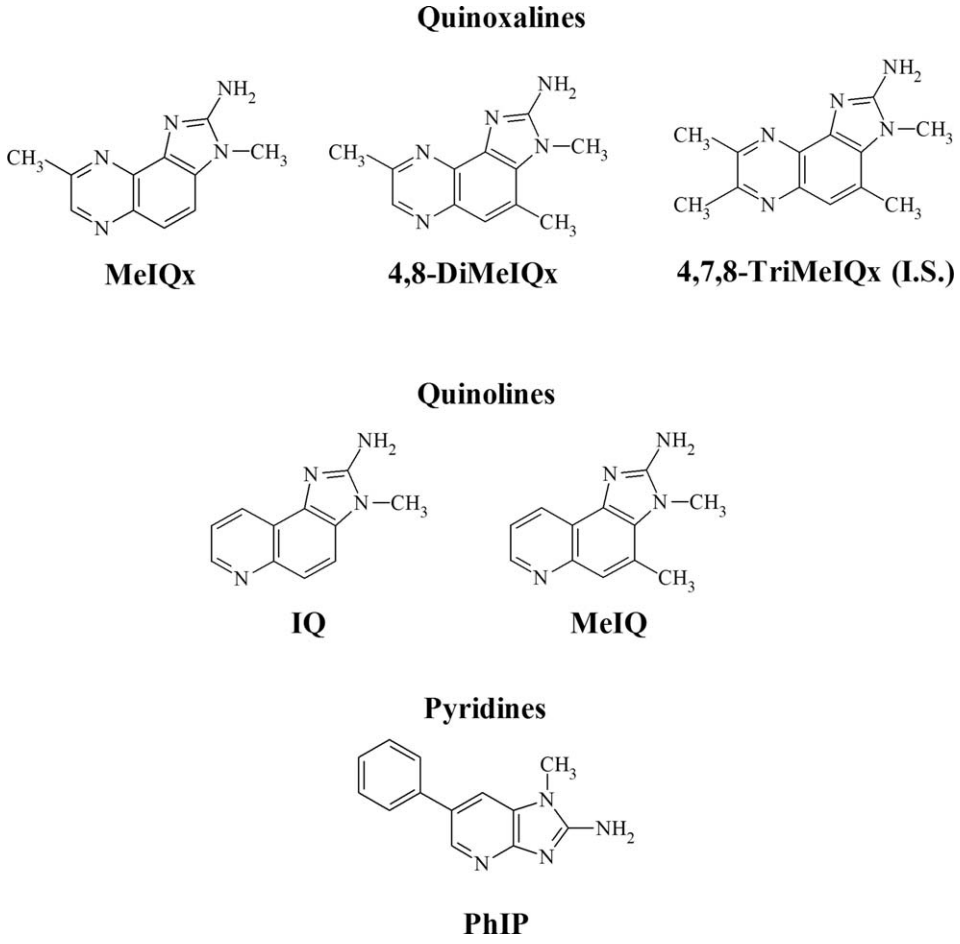

FIG. 1. STRUCTURES AND GENERAL ABBREVIATION OF INVESTIGATED HETEROCYCLIC AMINES

meat samples were thermally processed by applying different kinds of food condiments during the cooking process. The used food condiments are easily available and frequently using during the cooking of meat foods either in Saudi Arabia or worldwide.

The camel meats and food condiments (garlic, ginger, pepper, tomato and onion) were purchased from local supermarket in Riyadh, Saudi Arabia. The visible fats were removed from raw meat and prepared into fillets of 1.2 cm thickness. The fillets were fried on a nonsticky frying pan (Tafel, France) using an electric stove (Nippotec, China). In accordance with the European Union COST Action 99 – EUROFOODS, frying means food thermally processed on heated fat or oil. The cooking temperature range was between 210C and 220C, to monitor the cooking temperature of meat samples, five insulated-wire probes were applied at both the upper and lower sides of fillet; in fillet center; between fillet and pan, and in pan center. The meat samples cooking time was 4 min, 2 min each side of the fillets. After the cooking process, the samples temperature was allowed to come down at room temperature and then the weight loss of the cooked meat samples were determined. The weight loss was determined as the difference between the mass of the meats before and after food preparation procedure. A Microtron MB 800 food processor was used to ground the cooked meat samples followed by cataloging, bottling (amber flasks) and keeping (freezer) at –30C until SPE/UPLC-MS analysis.

Sample Extraction Procedure

The HAs extraction and purification from cooked camel meat samples were carried out by previous developed methods (Gross and Grüter 1992; Toribio *et al.* 2007). The frozen meat samples were removed from the freezer and allowed to come down at room temperature. Three grams of meat samples were homogenized with sodium hydroxide (1 M) using an Ultra-Turrax® T25 digital (IKA®, Staufen, Germany) blender followed by carefully mixing with 13 g of inert diatomaceous earth material. The meat sample was transferred to a blank Extrelut column attached to a Bond Elut PRS (500 mg) cartridge, the PRS cartridge was previously preconditioned with 15 mL HCl (0.1 M), 10 mL water and 5 mL

TABLE 1. MRM CONDITIONS USED WITH TRIPLE QUADRUPOLE (MS/MS) INSTRUMENT[a]

		Quantification		Confirmation[b]	
HAs	Precursor ion $[M+H]^+$ *m/z*	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	242	227	25	201	30
PhIP	225	210	25	183	30
IQ	199	184	30	157	35
MeIQ	213	198	25	197	30

[a] Dwell time was 0.025 s in all cases.
[b] Confirmation ion intensity was higher than 10%.

methanol, correspondingly. The ethyl acetate was used as extracting solvent and a volume of 75 mL was used to extract the HAs from inert diatomaceous earth material to the PRS cartridge. Once the ethyl acetate completely eluted through PRS cartridge, the cartridge was dried under vacuum and washed sequentially with 15 mL water/methanol (6:4, v/v) and 2 mL water. Afterward, the PRS cartridge was attached to a Bond Elut C_{18} (100 mg) cartridge, the C_{18} cartridge was formerly preconditioned with 15 mL methanol and 5 mL water, respectively. The HAs were desorbed from PRS cartridge to C_{18} cartridge using 20 mL ammonium acetate (0.5 M) adjusted at pH 8.5 with ammonia solution. Finally, the C_{18} cartridge was rinsed with 15 mL water (5 mL) followed by drying under vacuum. Once the C_{18} cartridge dried completely, the HAs were eluted to a 1.5 mL tube (Eppendorf, Wesseling-Berzdorf, Germany) using a solvent mixture of 0.8 mL methanol/ammonia (9:1, v/v). The solvent mixture containing HAs was evaporated using nitrogen stream followed by dissolving with 100 µL of methanolic solution containing 4,7,8–TriMeIQx (0.5 µg/g) internal standard. Finally, the solution was passed through 0.45 µm PTFE filter and refrigerated until UPLC-MS analysis.

The amount and recovery of HAs were determined by standard addition method, two nonspiked and three spiked camel meat samples at different levels (50%, 100% and 500%). The recoveries were calculated from the slope of linear regression achieved from the added HAs quantity to the calculated HAs quantity.

HAs Determination Method

HAs separation was performed on Waters Acquity® UPLC system outfitted by a quaternary pump (Milford, USA). Waters reversed phase analytical column (Acquity® BEH C_{18}, 50 mm x 2.1 mm id, 1.7 mm particle size) (Milford, USA) was used. A binary mobile phase acetonitrile (A) and 30 mM formic acid/ammonium formate, pH 4.75 (B) at flow speed of 1 mL min^{-1} were applied for HAs separation. The gradient mobile phase elution program was 0–0.1 min, 5% A; 0.1–1.5 min, 5–30% A; 1.5–1.8 min, 30–60% A; 1.8–2.4 min, 60% A; 2.4–2.5 min, come back to its original parameters; 2.5–3 min, column equilibration. The injection volume of the sample was 10 µL (Barcelo-Barrachina *et al.* 2006). The column was washed with a mixture of methanol:water (50:50, v/v) for 10 min after every 20 samples injection.

HAs detection was carried out by Micromass Quattro Premier triple quadrupole mass spectrometer (Milford, USA) equipped with electrospray ionization (ESI) source in Z-spray type configuration. The mass spectrometer system was operated in positive ionization mode and multiple reaction monitoring (MRM) mode was applied to record the sample data. The operational conditions of ESI source were as follows: cone voltage, 40 V; capillary voltage, 3.0 kV; source temperature, 100C; desolvation temperature, 400C; desolvation gas flow speed, 804 L/h; cone gas flow speed, 49 L/h. For cone gas, nitrogen generator model NM30LA (Peak Scientific, Inchinnan, UK) was used to obtain the nitrogen gas. The argon was used as collision gases. To supply the primary vacuum to the MS system, an Oerlikon rotary pump, model SOGEVACSV40 BI (Cedex, France) was used. Table 1 illustrates the MRM conditions used with triple quadrupole (MS/MS) instrument. (Barcelo-Barrachina *et al.* 2006). The Waters MassLynx V4.1 software (Milford, USA) was used to acquire the instrumental data.

RESULTS AND DISCUSSION

The results obtained from the analyzed camel meat samples are demonstrated in Table 2. The most frequent HAs MeIQx, 4,8-DiMeIQx and PhIP were detected in all of the analyzed camel meat samples. In control sample, the amounts of HAs were indentified at higher concentration, the HAs MeIQx (2.35 ng/g), 4,8-DiMeIQx (2.10 ng/g) and PhIP (5.22 ng/g), while HAs MeIQ and IQ were also identified but found below limit of quantification ($S/N = 10$). The presence of HAs in control sample are in concurrence with the results achieved by Khan *et al.* (2015a), who has

TABLE 2. Amount of heterocyclic amines in camel meat, thermally processed using natural food condiments

Sample	MeIQx (ng/g ± s[a])	4,8-DiMeIQx (ng/g ± s[a])	PhIP (ng/g ± s[a])	MeIQ (ng/g ± s[a])	IQ (ng/g ± s[a])
Camel meat[b]	2.35 ± 0.10	2.10 ± 0.12	5.22 ± 0.15	nq	nq
Camel with garlic	0.92 ± 0.06	0.78 ± 0.04	1.31 ± 0.13	nd	nd
Camel with ginger	0.74 ± 0.04	0.62 ± 0.04	1.79 ± 0.09	nd	nd
Camel with pepper	1.17 ± 0.08	1.10 ± 0.08	2.83 ± 0.11	nq	nq
Camel with tomato	0.55 ± 0.02	0.42 ± 0.02	1.10 ± 0.08	nd	nd
Camel with onion	0.98 ± 0.08	0.83 ± 0.07	1.85 ± 0.10	nd	nd

[a] Standard deviation achieved from the standard addition calibration.
[b] sample fried without food condiments (control); nq: below limit of quantification (signal-to-noise, 10:1); nd: not detected

investigated similar cooking procedure and temperature in camel meat samples. Nevertheless, in the present study the camel meat samples were thermally processed by various food condiments, the amount of HAs MeIQx, 4,8-DiMeIQx, and PhIP, were indentified comparatively at lesser level from 0.42 to 3.33 ng/g, whereas MeIQ and IQ were not detected in any of the analyzed meat samples. The outcomes from the studied samples revealed that the amounts of HAs diminish subsequent to the addition of food condiments (Table 2). The HAs detection limits and recoveries in each sample are illustrated in Table 3. The detection limits were obtained between 0.01 and 0.05 ng/g, nonetheless, recoveries were achieved up to 60%, depending on the types of HAs and samples. The obtained detection limits and recoveries values are in agreement as formerly determined in cooked beef samples (Toribio *et al.* 2007). This concurrence can be because of similar meat composition of camel meat (Khan *et al.* 2015a) and identical extraction SPE procedures with the exemption of extraction solvent dichloromethane was applied as a substitute of ethyl acetate (Toribio *et al.* 2007). To display the results, as an example the UPLC-MS/MS chromatograms of HAs in camel meat cooked with pepper are illustrated in Fig. 2, it can be observed that outstanding sensitivity was achieved in the analysis, even though six MRM transitions were obtained at the same time. Concurring with the achieved outcomes in such types of cooked meat products, MeIQ and IQ are not very commonly detected, this fact maybe because of the high thermal cooking temperatures is required for their occurrence (Arvidsson *et al.* 1997). Even with the truth, such HAs have been detected at amount (<1.0 ng/g) in the cooked meats thermally processed in a same way than those achieved in the present study (Busquets *et al.* 2004). On the other hand HAs MeIQx and 4,8-DiMeIQx are usually occurred at concentration (>0.1 ng/g) at cooking temperatures from 200C to 210C (Khan *et al.* 2009b). Relating to the HAs PhIP, it is normally detected at concentration (>5.0 ng/g) which were found in the comparable range to the concentration identified in the earlier studied cooked meats (Puangsombat *et al.* 2012). However, in some other protein-rich food for instance swordfish, the concentration of PhIP was identified up to 121 ng/g (Khan *et al.* 2013). These amounts are superior to those usually indentified in cooked chicken meat (27.5 ng/g), which is characteristically described as one of the most contaminated food item (Khan *et al.* 2009b). The PhIP has been assumed to be as one of the major exposure to the intake of HAs in our daily life (Keating and Bogen 2004).

Table 2 illustrates the effects of various food condiments on the formation and diminution of HAs in fried camel meat. As compared to the control sample, the amounts of HAs were identified at lower concentration in camel meat samples cooked with various food condiments. The identified HAs were MeIQx, 4,8-DiMeIQx and PhIP, whereas, MeIQ and IQ were not detected in any of the analyzed samples. The obtained outcomes revealed that the cooking by a

TABLE 3. Detection limits (DL, ng/g)[a] and recoveries (*R*, %) of HAs in camel meat cooked with natural food condiments

	MeIQx		4,8-DiMeIQx		PhIP		IQ		MeIQ	
Sample	DL	*R*	DL	*R*	DL	*R*	DL	*R*	DL	*R*
Camel meat[b]	0.02	63	0.02	65	0.01	58	0.01	59	0.02	61
Camel with garlic	0.03	58	0.04	55	0.05	52	0.03	56	0.02	58
Camel with ginger	0.04	60	0.04	57	0.03	53	0.03	56	0.03	58
Camel with pepper	0.03	62	0.03	65	0.02	57	0.02	57	0.03	58
Camel with tomato	0.05	56	0.05	55	0.04	54	0.05	54	0.04	56
Camel with onion	0.03	60	0.03	58	0.01	58	0.01	57	0.01	57

[a] Detection limits, signal-to-noise ratio of 3:1.
[b] Sample fried without food condiments (control).

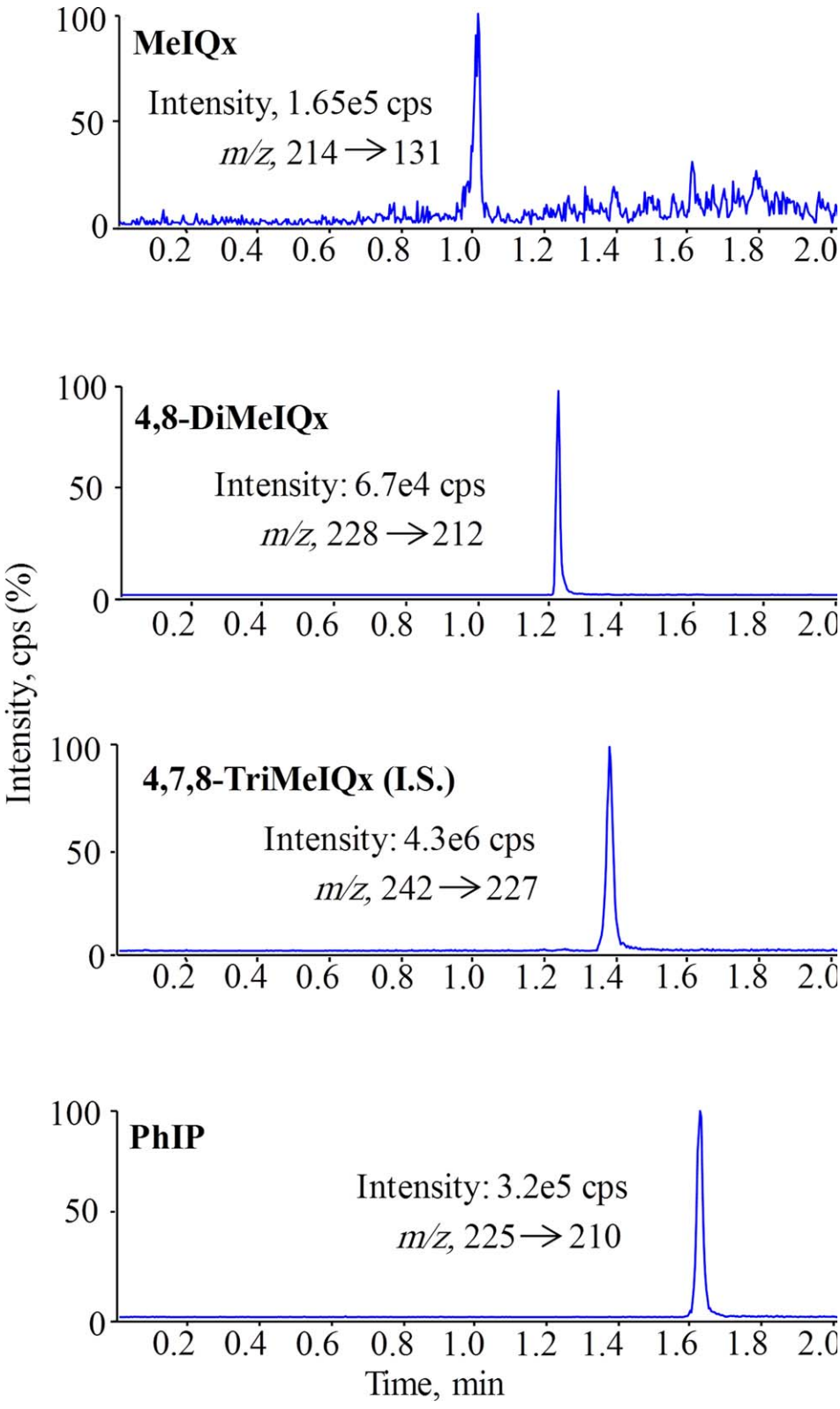

FIG. 2. UPLC-MS/MS CHROMATOGRAMS OF HETEROCYCLIC AMINES IN COOKED CAMEL MEAT (PEPPER)

variety of condiments was capable to diminish the HA occurrence more efficiently. The camel meat thermally processed with tomato has showed the higher inhibitory effect on the formation of HAs. Vitaglione *et al.* (2002) have described that the carotenoid-rich foods for instance tomato reduced the occurrence of HAs either in meat juice model system or chemical model system, having glucose, glycine and creatine as precursors. In meat juice model system, using tomato flavonoids (Quercetin and carotenoid) have exhibited the inhibition of HAs occurrence up to 67% (Vitaglione *et al.* 2002). However, in chemical model system the extract of these flavonoids at certain levels has exhibited the inhibition of HAs occurrence up to 36% (Vitaglione *et al.* 2002). The obtained results in current study are found in concurrence as compared with the previous studies (Vitaglione *et al.* 2002). The camel meat thermally processed with garlic, ginger and onion have also demonstrated a high inhibition on the HAs formation (Table 2). These defensive influences have been ascribed because of the various antioxidant substances (flavonoids and polyphenolics,) occur in such type of condiments (Murkovic *et al.* 1998). Many authors have revealed that plants belong to *Allium* family shows the high antioxidant properties (Nuutila *et al.* 2003; Shona *et al.* 2004; Stoilova *et al.* 2007). Garlic, ginger and onion are the main sources of nutritional flavonoids in several countries. The diallyl trisulphide, diallyl disulphide and allicin are the major antioxidative substances found in garlic (Nuutila *et al.* 2003). Nevertheless, gingerol and quercetin are the two most important antioxidative substances found in ginger and onion, respectively (Nuutila *et al.* 2003; Stoilova *et al.* 2007). Relatively, the meat sample cooked with pepper does not prove any high diminution on the occurrence of HAs and the achieved concentrations were 50% to the amounts as attained in the control meat sample. The low inhibition source possibly because of the radical scavenging properties of numerous condiments might have obstructed in the occurrence of HAs (Murkovic *et al.* 1998). Consequently, the camel meat cooked by a variety of food condiments illustrates the capability to diminish the HAs occurrence. Such information may be elucidated because of the presence of antioxidants which could have pro-oxidative effects with the subsequent formation of peroxyl radicals or by the scavenging of oxygen or free radicals (Johansson and Jägerstad 1996; Balogh *et al.* 2000; Vitaglione and Fogliano 2004; Gibis and Weiss 2012;Zhang *et al.* 2013; Khan *et al.* 2015b; Viegas *et al.* 2015). Nonetheless, in the present study, the applied thermal treatment conditions for the processing of camel meat are not so simple to assess with the previous works because of several authors have applied a mixture of various food condiments together with different cooking parameters (Lan *et al.* 2004; Gibis 2007; Khan *et al.* 2009a). Moreover, many authors have also described the utilization of merely one kind of food condiments with different cooking parameters for the diminution of HAs in meat products (Persson *et al.* 2003; Melo *et al.* 2008; Oz and Kaya 2011). To evaluate the individual's exposure to the HAs, it required to judge the kinds of meat, cooking process and degree of doneness in epidemiological studies.

CONCLUSIONS

This is the first study relating to the inhibition of HAs by means of some common food condiments in cooked camel meat. In this work, five HAs such as MeIQx, 4,8-DiMeIQx, PhIP, MeIQ and IQ have been studied in camel meat samples thermally processed with or without food condiments. Our results evidently demonstrate that the formation of HAs in cooked camel meat is highly affected by using food condiments. Relatively, pepper has supplied the highest amounts of HAs as compared with other condiments for instance tomato, garlic, ginger and onion. Such information may be explained because of the presence of antioxidants which could have pro-oxidative effects with the subsequent formation of peroxyl radicals or by the scavenging of oxygen or free radicals. The current study also illustrates that the concentrations of HAs can be kept at low levels by cooking the meat that include food condiments. The obtained results could be used to estimate the human intake of HAs either in Saudi Arabia or global and supplied to the search of good food condiments that reduce the threat of exposure to HAs, and thus to advance the food security and quality.

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