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Occurrence of heterocyclic amines in several home-cooked meat dishes of the Spanish diet

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Abstract

Heterocyclic amines (HAs) were determined in several of the most frequently eaten meat dishes in Spain such as fried beef hamburger, fried pork loin, fried chicken breast, fried pork sausages, griddled chicken breast, griddled lamb steak and griddled beef steak. All of the products tested were household cooked. The HAs were analysed in the selected meat dishes using an analytical method based on solid-phase extraction followed by liquid chromatography coupled to tandem mass spectrometry. DMIP, MeIQx, 4,8-DiMeIQx, Norharman, Harman, PhIP, Trp-P-1, AαC and MeAαC were the amines most frequently found at concentrations of up to 47 ng g^{-1} of cooked meat. Glu-P-2, IQ, MeIQ, Glu-P-1, 7,8-DiMeIQx and Trp-P-2 were only found in a few of the meat dishes and their concentrations were lower than 1 ng g^{-1} of cooked meat. The highest amounts of HAs, especially PhIP and DMIP, were formed in fried chicken breast and the lowest were formed in fried beef hamburger and in fried pork sausages. Daily intake of HAs in Spain was estimated at 606 ng of mutagenic HAs per capita and day, DMIP and PhIP being the main contributors.

Keywords: Food analysis; Heterocyclic aromatic amines

1. Introduction

In the Spanish diet, meat and/or fish are commonly present. These foodstuffs are cooked in multiple ways (such as frying and griddling) that provide suitable sensorial properties. Nevertheless, some of these cooking methods create temperatures high enough (125–250 °C) that favour the formation of mutagenic compounds such as heterocyclic amines (HAs) [1–4]. Although HAs are formed at ppb levels (ng g^{-1}), their intake may be dangerous for human health because of the total amount of cooked meat consumed. A prospective study of diet developed in Spain based on consumption data registered in 1999, showed that 65 kg of meat and 31 kg of fish were the Spanish per capita consumption in that year, bovine, poultry and pork being the most consumed meats in the total of Spanish regions [5].

At present, more than 20 HAs have been isolated and characterised from different cooked food samples and several model systems [6]. In view of their chemical structure, they can be classified into two main groups called IQ-type HAs or aminoimidazoazaarenes, and non-IQ-type HAs or carbolines. Those of the first type are produced by Maillard reaction when mixtures of creati(ni)ne, amino acids and sugars are heated [7]. Those of the second type are mainly formed by the pyrolysis of amino acids and proteins at higher temperatures than the IQ-type HAs [8]. The variety and amounts of HAs depend on the cooking temperature, duration of the cooking process and the concentration of HAs precursors and compounds with enhancing or inhibiting effects, with temperature being a critical parameter in HAs formation [3,7,9–12].

Concerning their toxicity, HAs have been tested for their mutagenic activity in assays in vitro and in vivo with positive results for most HAs [13–16]. In particular, the amines called IQ and MeIQ belong to a class of supermutagens, some others present lower mutagenicity, and neither Harman nor Norharman is mutagenic but instead strengthen the genotoxicity of other HAs [17]. In 1993, the International Agency for Research and Cancer, IARC, judged the heterocyclic amines MeIQ, MeIQx and PhIP to be possible human carcinogens and IQ as probably carcinogenic [18].

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The risk of chronic exposure to potentially carcinogenic compounds through diet depends on the dose, frequencies of exposure to each compound and individual genetic susceptibilities [19–21]. In order to evaluate dietary exposure to HAs, accurate assessment of individual food consumption is essential. For this purpose, it is necessary to develop food frequency questionnaires where people are asked about the type and amount of meat, poultry, fish and gravy ingested, frequency of consumption, cooking method, degree of doneness and browning. Information obtained from food frequency questionnaires, together with the corresponding HAs content in the different diet items, would allow determination of the HAs intake based on a normal diet. In recent years, dietary intakes of several HAs in some populations have been published [22–25].

The present study attempts to identify and quantify HAs levels in some of the most popular meat dishes in Spain. The studied dishes were selected from information on dietary practices, such as the way of cooking or individual preferences for the level of meat doneness. These parameters were previously obtained by combining the data of several food frequency questionnaires. Fried beef hamburger, fried pork loin, fried chicken breast, fried pork sausages, griddled chicken breast, griddled lamb steak and griddled beef steak were the meat dishes selected. Accurate determination of HAs in the cooked samples was performed using the solid-phase extraction method described by Gross and Grüter [26] modified by Galceran et al. [27] and liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS) [28]. It was necessary to use such a selective and sensitive detection system because of the low levels of HAs formed and also to prevent interferences from the complex sample matrix [29,30]. Together, the information on Spanish eating habits obtained from food frequency questionnaires and the analytical HAs contents on the selected meat dishes made it possible to estimate the intake of HAs in Spain.

2. Experimental

2.1. Chemicals

Solvents and chemicals were of HPLC or analytical grade, and the water was purified in an Elix-Milli-Q system (Millipore, Bedford, MA, USA). All the solutions were passed through a 0.45 μm filter and the sample purified fractions were passed through a 0.22 μm filter before injection into the HPLC system.

The studied compounds were 2-amino-1,6-dimethylimidazo[4,5-*b*]pyridine (DMIP), 2-amino-3-methylimidazo-[4,5-*f*]quinoline (IQ), 2-amino-3,4-dimethylimidazo[4,5-*f*] quinoline (MeIQ), 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), 2-amino-8-methyl-3-trideuteromethylimidazo[4,5-*f*]quinoxaline (D_3-MeIQx), 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-6-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazole (Glu-P-1), 2-aminodipyrido[1,2-*a*:3′,2′-*d*]imidazole (Glu-P-2), 2-amino-3,4,7,8-tetramethylimidazo[4,5-*f*]quinoxaline (TriMeIQx), 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-1), 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-2), 2-amino-9*H*-pyrido-[2,3-*b*]indole (AαC), 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole (MeAαC), purchased from Toronto Research Chemicals (Toronto, Canada), and 1-methyl-9*H*-pyrido-[4,3-*b*]indole (Harman), 9*H*-pyrido-[3,4-*b*]indole (Norharman), from Sigma (St. Louis, MO, USA). Stock standard solutions of 130 μg g^{-1} in methanol were prepared and used for further dilution. Both TriMeIQx and D_3-MeIQx were used as internal standards.

Empty Extrelut-20 extraction cartridges were provided by Merck (Darmstadt, Germany), and Isolute diatomaceous earth refill material was obtained from IST (Hengoed, UK). Propylsulfonate silica PRS (500 mg) cartridges and end-capped Bond Elut C_{18} (100 and 500 mg) cartridges were from Varian (Harbor City, USA). These cartridges were preconditioned with dichloromethane (7 ml) for PRS, and methanol (5 ml) and water (5 ml) for C_{18}.

Raw minced beef, beef steaks, lamb steaks, pork loin, pork sausages and chicken breasts as well as ingredients such as salt or olive oil were purchased at a local supermarket in Barcelona.

2.2. Instrumentation

An Agilent Technologies (USA) model Series 1100 equipped with a quaternary pump system and an autosampler was coupled to an API 3000™ (Perkin-Elmer Sciex, Canada) provided with a Turbo Ionspray™ ionization source and a triple quadrupole analyzer. Data were acquired with Analyst 1.1 software.

A Supelco Visiprep and a Visidry SPE vacuum manifold (Supelco, Gland, Switzerland) were used for manipulations with solid-phase extraction cartridges and solvent evaporation, respectively.

A Testo surface thermometer (Testo Instruments, Cabrils, Spain) was used for temperature monitoring.

An Ultra-Turrax® T 25 basic (IKA, Staufen) was used to homogenise cooked meats.

2.3. Analytical conditions

The chromatographic separation of HAs was carried out using a microbore reversed phase column Symmetry® C_8 (150 mm × 2.1 mm) with a particle size of 5 μm (Waters, Milford, USA). Optimal separation was achieved with a binary mobile phase at a flow-rate of 0.3 ml min^{-1}. Solvent A was acetonitrile and solvent B was a 30 mM acetic acid–ammonium acetate buffer adjusted to pH 4.5. The elution program was: 0–0.5 min, 5% A; 0.5–15 min, 5–20% A; 15–18 min, 20–60% A; 18–24 min, 60% A; 24–27 min,

Table 1
MRM parameters used with the triple quadrupole instrument

Analyte	Precursor *m/z*	Quantitation precursor → product ion (*m/z*)	Confirmation precursor → product ion (*m/z*)	Collision offset voltage (V)
DMIP	163	163 → 148	163 → 147	37
Glu-P-2	185	185 → 158	185 → 168	37
IQ	199	199 → 184	199 → 157	39
MeIQx	214	214 → 199	214 → 173	38
D_3-MeIQx	217	217 → 199	217 → 173	38
MeIQ	213	213 → 198	–	38
Glu-P-1	199	199 → 172	199 → 182	37
7,8-DiMeIQx	228	228 → 213	228 → 187	40
4,8-DiMeIQx	228	228 → 213	228 → 187	40
Norharman	169	169 → 115	–	49
TriMeIQx	242	242 → 227	242 → 201	38
Harman	183	183 → 115	183 → 168	49
Trp-P-2	198	198 → 181	198 → 154	35
MeAαC	198	198 → 181	198 → 154	35
Trp-P-1	212	212 → 195	212 → 168	36
PhIP	225	225 → 210	–	43
AαC	184	184 → 167	184 → 140	38

Interchannel time delay: 5 ms; in all cases dwell time was 150 ms.

return to the initial conditions; 5 min equilibration. In all cases the volume of injection was 5 μl [28].

Optimal ionisation source working parameters for monitoring heterocyclic amines were: spray voltage, 2.5 kV; nebulizer gas 11 a.u.; curtain gas, 14 a.u.; turboionspray gas flow-rate 7000 a.u., turboionspray gas temperature, 450 °C; declustering potential, 30 V. Data acquisition was performed in multiple reaction monitoring (MRM) mode using the protonated molecular ion as precursor ion. Table 1 gives MRM conditions such as collision offset voltage, dwell time and mass-to-charge corresponding to the precursors and product ions. The most abundant product ion was monitored for HAs quantitation, and the second most abundant product ion was monitored in order to confirm HAs identification.

2.4. Sample treatment

Raw meats were purchased at a local supermarket and kept at 4 °C until cooking. Skin, fat parts and bones were removed from chicken breast, pork loin and lamb steak, before cooking. The minced beef was made into hamburger paddies prior to cooking and testing.

The cooking methods selected were frying and griddling. According to the Eurocode descriptor system (COST Action 99/EUROFOODS), frying means cooking in heated oil or fat, which then become an ingredient of the finished product. Griddling means cooking on a heated flat metal surface. A small amount of fat or oil may be used to grease the metal surface. In our experiments, olive oil that had an acidity index of 0.4° was used and meat was seasoned with salt. All meat dishes were cooked on a gas cooker and the temperature of the pan or the griddle pan was monitored with a surface thermometer. A Teflon-coated frying pan (260 mm × 260 mm) and an enamelled cast iron griddle (240 mm × 290 mm) were used.

All meat samples were cooked to the degree of doneness and browning that the participants in a food frequency questionnaire preferred. The cooked outer layer (2–3 mm thick) of the meat was peeled-off and ground. Finally, the meat samples were stored at −18 °C until analysis. Cooking methods, temperatures, times, weights of the meat and seasoning are detailed in Table 2.

A standard addition method, four spiked and two non-spiked samples, was used to quantify HAs in the food

Table 2
Description of the food processing

Meat type	Raw meat (g)	Thickness (raw meat) (cm)	Type of preparation	Cooked meat (g)	Cooking temperature (°C)	Cooking time (min per side)	Meat surface (g)	salt (g/steak)	olive oil (g)
Beef hamburger	732.7	0.8	Fried	483.5	175–200	5.6	178.9	1	21.1
Pork loin	678.3	0.5	Fried	430.4	175–200	5.0	257.6	1	29.5
Chicken breast	614.4	0.8	Fried	379.0	175–200	6.0	146.3	1	20.0
Pork sausages	1117.6	2	Fried	942.5	175–200	4.5	148.9	1	14.3
Chicken breast	496.8	0.8	Griddled	361.4	175–200	6.5	88.7	1	11.5
Lamb steak	475.4	0.8	Griddled	418.2	175–200	5.5	105.8	1	–
Beef steak	350.0	0.5	Griddled	208.0	180–210	2.0	208.0	1	–

matrices. HAs from the cooked meat surface were extracted and purified by the method developed by Gross and Grüter [26] and modified by Galceran et al. [27]. Briefly, 3 g of ground surface samples were mixed with 6 ml of 1 M NaOH and homogenised in an Ultra-Turrax®. Next, homogeneous samples were mixed with diatomaceous earth and extracted with dichloromethane. The eluate was passed through PRS and C_{18} columns. The method yielded two fractions (polar and less polar HAs) that were evaporated to dryness under a stream of nitrogen. The final extracts were dissolved in a suitable volume of a solution of methanol–mobile phase (50:50) that contained internal standards. Then samples were filtered through a 0.22 μm membrane filter. Finally, the purified meat extracts were injected into the LC–MS system.

3. Results and discussion

3.1. Selection of the most consumed meat dishes

Detailed and representative information on Spanish eating habits is necessary to have a realistic knowledge of HAs intake. Basic information for the selection of some of the most popular meat dishes of the Spanish diet was obtained from a prospective investigation about eating habits developed by the EPIC project (European Prospective Investigation into Nutrition Cancer and Health) [31]. The food questionnaire was given to 3221 middle-aged men and women from five representative regions of Spain who agreed to participate in the prospective cohort and to have their health status followed up for the rest of their lives. EPIC participants were inquired about the food they had eaten during the last 24 h, specifying the cooking method and the amount of the consumed food. In order to quantify the amount of food consumed for each dish, some photos of different dishes and amounts were shown to the participants. Although the EPIC questionnaire provides valuable knowledge about food consumption, information about cooking methods and the most frequently consumed animal parts is not available. In order to achieve additional information and details about the most usual cooking conditions, another approach to household habits was performed in Barcelona (Catalonia, Spain) in 2002 [32]. This local food frequency questionnaire was answered by 459 persons, who were asked about their household eating habits such as cooking method; use of fats; use of cooking residues to make gravies; consumption of meat, meat skin, poultry and fish; weight and frequency of consumption. In addition, participants indicated their preference for degree of surface browning and meat doneness by looking at photos of meat cooked at different temperatures. From the answers to both food frequency questionnaires several of the most consumed meat dishes in Spain were selected for this study. The selected meat dishes were: fried beef hamburger (15.5 g), fried chicken breast (6.6 g) fried pork loin (5.4 g), fried pork sausages (5.4 g), griddled beef steak (2.3 g), griddled lamb steak (0.4 g) and griddled chicken breast (0.3 g). The data in brackets corresponds to the daily intake per capita of each selected food item obtained from the EPIC study. Although griddled lamb steak and griddled chicken breast did not figure among the most consumed meat dishes in Spain, they have been considered in this work because they were found to be frequently consumed in Barcelona [32].

3.2. HAs determination

One of the aims of this study was to quantify HAs formed in some meat dishes cooked under normal household conditions. For this reason, the selected foods were processed at cooking conditions similar to the household habits previously described (Table 2). For sample preparation, only the outer layer of the meat sample was analysed since HAs are mainly present in the crust. Then, the amounts of HAs found in the crust were converted into amounts of HAs in the whole cooked meat dish by taking into account the ratio between weight of crust and weight of the entire meat dish. Quantitation was performed by standard addition in order to overcome matrix effects. In general, significant variations in HAs recoveries were observed depending on the cooked meats analysed. For example, while recovery for 4,8-DiMeIQx attained 82% in griddled chicken breast, a lower recovery, 46%, was obtained when analysing griddled beef steak. In the cooked meats recoveries of HAs ranged between 15 and 99%, DMIP and PhIP being the ones with minor recoveries. The results obtained in the analysis of HAs and their corresponding standard deviations obtained from a five-level standard addition calibration are given in Table 3. In some cases, when a signal-to-noise ratio lower than 10 was obtained, the results were expressed as lower than the limit of quantitation. The limit of quantitation was estimated from the signal-to-noise ratio measured when the lowest spiked sample was analysed. Fig. 1 shows a chromatogram corresponding to a purified sample of unspiked griddled chicken breast. It can be seen that HAs were present in a wide range of concentrations; while high levels of some analytes were formed, such as Norharman and PhIP, others were present to a lesser degree for example MeIQ and MeAαC. In these cases their concentrations were expressed in terms of the estimated limit of quantitation.

Generally, DMIP, MeIQx, 4,8-DiMeIQx, Norharman, Harman, PhIP, Trp-P-1, AαC and MeAαC were the HAs most frequently found in the analysed meat dishes, while Glu-P-2, IQ, MeIQ, Glu-P-1, 7,8-DiMeIQx and Trp-P-2 were only found in a few of them. DMIP, PhIP and Norharman were formed in relatively high amounts, their concentrations being <0.2–30, <0.2–47 and 0.3–41 ng g^{-1} cooked weight, respectively. In contrast, the other HAs were formed at concentrations lower than 8 ng g^{-1}. The highest amount of PhIP was detected in fried chicken breast in agreement with the literature [33,34]. The highest amount of DMIP, which is a compound not currently analysed in food, was also formed in fried chicken breast in accordance with data

Table 3
Concentration of HAs in cooked meat dishes and their corresponding intake

HAs	Fried beef hamburger		Fried pork loin		Fried chicken breast		Fried pork sausages		Griddled chicken breast		Griddled lamb steak		Griddled beef steak	
	Content (ng g^{-1} ± s[a])	Intake (ng of Ha[b])	Content (ng g^{-1} ± s[a])	Intake (ng of Ha[b])	Content (ng g^{-1} ± s[a])	Intake (ng of Ha[b])	Content (ng g^{-1} ± s[a])	Intake (ng of Ha[b])	Content (ng g^{-1} ± s[a])	Intake (ng of Ha[b])	Content (ng g^{-1} ± s[a])	Intake (ng of Ha[b])	Content (ng g^{-1} ± s[a])	Intake (ng of Ha[b])
DMIP	<0.2	<3.1	3.9 ± 1.1	20.9	29.7 ± 2.8	194.6	<0.5	<2.7	1.9 ± 0.1	0.6	ND	–	ND	–
Glu-P-2	ND	–	ND	–	ND	–	ND	–	ND	–	ND	–	ND	–
IQ	ND	–	<0.1	<0.5	ND	–	ND	–	ND	–	ND	–	ND	–
MeIQx	0.7 ± 0.1	11.0	1.9 ± 0.9	10.3	ND	–	ND	–	0.3 ± 0.1	0.1	1.3 ± 0.3	0.6	2.9 ± 0.4	6.7
MeIQ	ND	–	ND	–	ND	–	<0.4	<2.2	<0.1	<0.03	ND	–	ND	–
Glu-P-1	ND	–	<0.04	<0.2	ND	–	ND	–	ND	–	ND	–	ND	–
7,8-DiMeIQx	<0.04	<0.6	0.4 ± 0.3	2.2	ND	–	ND	–	<0.04	<0.01	ND	–	ND	–
4,8-DiMeIQx	<0.1	<1.5	0.5 ± 0.2	2.7	0.8 ± 0.3	5.2	ND	–	0.4 ± 0.1	0.2	1.8 ± 0.9	0.8	1.1 ± 0.1	2.5
Norharman	0.8 ± 0.1	12.4	2.3 ± 0.2	12.2	15.1 ± 0.2	99.2	0.3 ± 0.05	1.8	3.1 ± 0.04	0.9	9.1 ± 0.5	4.0	41.2 ± 7.4	95.0
Harman	1.9 ± 0.6	29.0	1.4 ± 0.5	7.4	7.5 ± 0.3	49.0	0.3 ± 0.04	1.5	1.1 ± 0.1	0.4	7.2 ± 0.4	3.2	5.3 ± 0.8	12.2
Trp-P-2	<0.1	<1.5	<0.4	<2.2	ND	–	ND	–	ND	–	<0.1	<0.03	ND	–
Trp-P-1	<0.05	<4.6	<0.05	<0.3	<0.2	<1.3	ND	–	ND	–	<0.1	<0.03	0.6 ± 0.1	1.4
PhIP	0.6 ± 0.02	8.8	2.5 ± 0.3	13.3	46.9 ± 2.1	307.0	<0.2	<1.1	2.3 ± 0.5	0.7	5.8 ± 0.2	2.6	4.8 ± 0.6	11.1
AαC	<0.04	<0.6	0.2 ± 0.01	0.9	<1.0	<6.6	ND	–	0.2 ± 0.2	0.1	0.5 ± 0.3	0.2	0.5 ± 0.1	1.2
MeAαC	<0.1	<1.5	<0.1	<0.5	ND	–	ND	–	<0.02	0.01	<0.2	<0.06	0.4 ± 0.04	0.9
Total intake[c]		75		74		663		9		3		11		131

ND: not detected.

[a] Standard deviation obtained from the addition standard calibration.

[b] Per day and person.

[c] Including HAs values below the limits of quantitation.

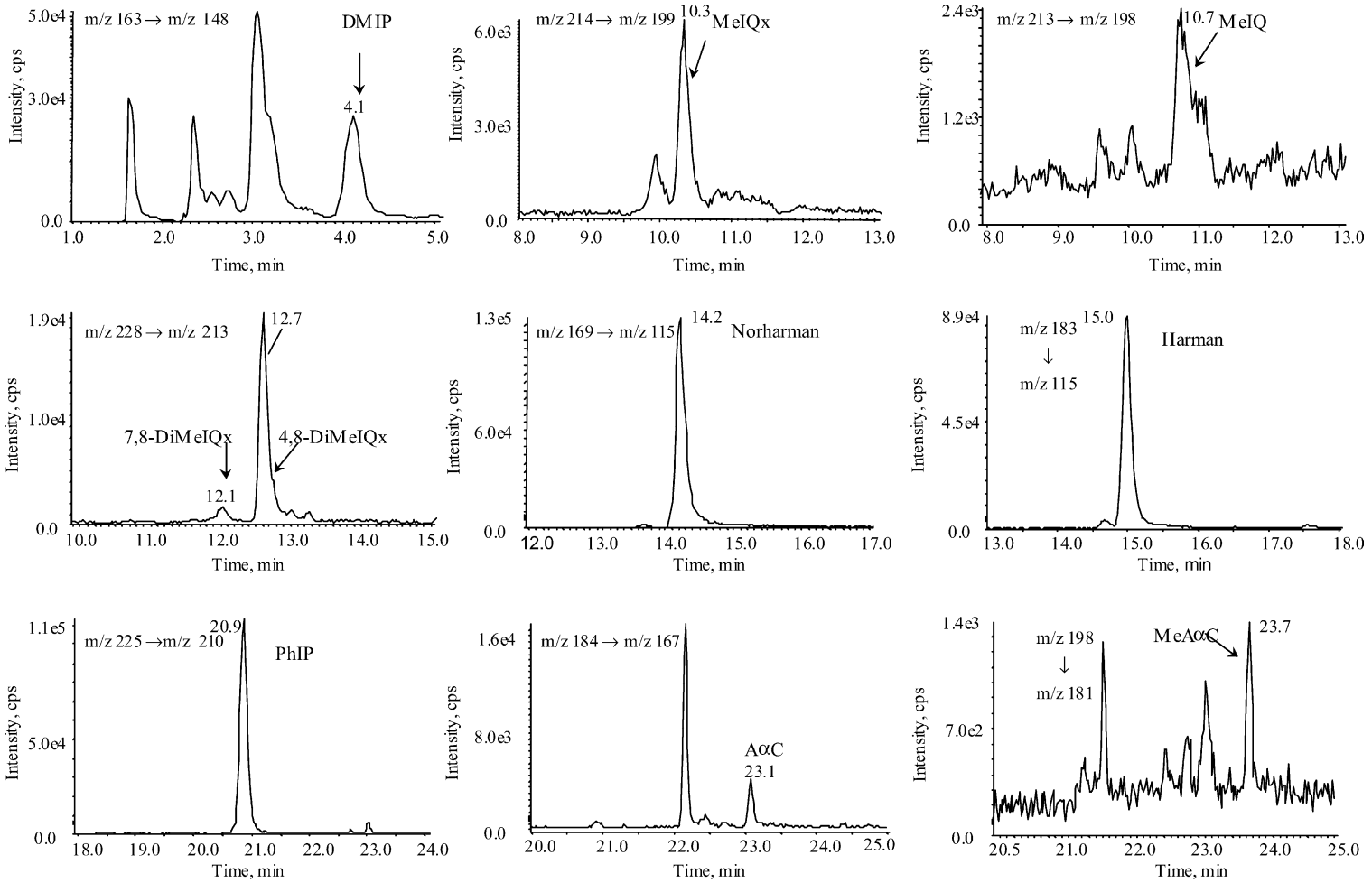

Fig. 1. Chromatograms of HAs from a sample of griddled chicken breast obtained with LC–MS/MS.

published by Knize and co-workers and in fried Norwegian minced meat product [35,36].

If the total amount of HAs is considered, the studied cooked meat dishes can be arranged in order, from the most contaminated to the least, as explained below. Concentrations of the comutagens Harman and Norharman were not included in this evaluation. Fried chicken breast was the dish with the highest concentration of HAs (77 ng g^{-1}). This concentration decreased up to 10 ng g^{-1} in griddled beef steak, fried pork loin and griddled lamb steak. Griddled chicken breast (5 ng g^{-1}) and fried beef hamburger (1 ng g^{-1}) contained lower amounts. Fried pork sausages seem to be the healthiest dish with regard to HAs occurrence since they were always found at unquantifiable concentrations. The low HAs amounts found in hamburgers and sausages could be explained by the presence of ingredients and additives that probably inhibit HAs formation in the cooking process. In this context, it has been reported that sulphur dioxide, which is frequently used as a preservative in sausages, is an inhibitor of the Maillard reaction [37].

3.3. HAs intake

From the HAs content in cooked meat dishes obtained and from information on eating habits provided by the food frequency questionnaires discussed above in Section 3.1, the HAs intake corresponding to the consumption of the studied meals was calculated. HAs concentrations in each analysed meat dish and their estimated intake in Spain is given in Table 3. Although in the EPIC study different products of the same type of cooked meat were not distinguished (i.e., fried pork sausages from fried pork loin), in this work the corresponding intake value was calculated on the basis that the meal which contained the highest amounts of HAs was the main source of intake. Mean daily intake of mutagenic HAs corresponding to these products are given for each meat dish in Table 3, expressed as ng of HAs per capita and day. It must be mentioned that the highest contribution to HAs intake corresponded to fried chicken breast (507 ng). Estimated mean value calculated without comutagens Harman and Norharman was 606 ng per capita and day. This, increased to 934 ng per capita and day if those comutagens were included. Results of HAs obtained from seven of the most consumed meat dishes in Spain were lower than those previously reported in Japan in 1985, where approximately 100,000 ng per capita and day was the estimated value [38]. These data are in acceptable agreement with those estimated in the USA in 1995 and 2001: 1690 ng per capita and day [39] and 455 ng per capita and day [23], respectively. Lower average intake values were estimated in recent published studies in Sweden, Switzerland and Japan, where 160 ng per capita and day [22], 330 ng per capita and day [24], and 72 ng per capita and day [25] were estimated. Note that intake values from the literature referring to daily intake per kg of body mass were converted to daily intake per capita considering a body mass of 65 kg in order to obtain comparable results. This first evaluation of HAs exposure showed that fried chicken breast seems to be the main source of HAs in the Spanish diet. Additional work to study the influence of different types of chicken is being carried out.

4. Conclusions

The analysis of seven of the most frequently eaten meat dishes in Spain showed that a wide variety of HAs was formed during the cooking process. A significant influence of cooked meat matrix on HAs determination was observed, indicating that standard addition calibration is mandatory to obtain reliable results. DMIP, MeIQx, 4,8-DiMeIQx, Norharman, Harman, PhIP, Trp-P-1, AαC and MeAαC were the most frequently found amines, while Glu-P-2, IQ, MeIQ, Glu-P-1, 7,8-DiMeIQx and Trp-P-2 were only present in a few of the studied meals. Moreover, DMIP, which is not usually analysed in meat, was present in most of the dishes. Generally, concentrations of HAs in the studied cooked meat dishes were lower than 8 ng g^{-1}, with the exception of DMIP, PhIP and Norharman which were found at high concentrations <0.2–47 ng g^{-1} on a cooked weight basis. The highest amounts of HAs, specially PhIP and DMIP, were formed in fried chicken breast, and the lowest were found in fried beef hamburgers and in fried pork sausages, where most HAs were found below the limit of quantitation.

The contribution of HAs intake in the studied dishes was estimated as 606 ng of mutagenic HAs per capita and day, with DMIP and PhIP being the main contributors to this value. The study of other dishes of the Spanish diet is in progress in order to obtain a realistic estimation of HAs intake. These future analytical results will provide useful values to epidemiologists for the determination of representative exposure of the Spanish population to HAs.

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