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Mutagenic heterocyclic amine content in thermally processed offal products

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ABSTRACT

Heterocyclic amines (HAs) are potent mutagens formed during heat-processing of proteinaceous food. PhIP is the most ubiquitous and abundant mutagenic HA. In this study several offal products (beef liver, lamb kidney and beef tongue) have been thermally processed and analysed for HAs for the first time. Norharman and harman were the amines most abundant, found at concentrations below 2 ng g^{-1}. PhIP was only formed up to 0.12 ng g^{-1}. Among the rest of HAs analysed, only DMIP, MeIQx and 4,8-DiMeIQx were detectable in cooked kidney and tongue up to 0.25 ng g^{-1}. The influence of cooking additives on HAs formation was evaluated, finding higher levels of norharman and harman, up to 8.87 ng g^{-1}, in liver processed with additives, whereas similar levels of the rest of HAs were found in these samples. The low amounts of HAs found after the prolonged thermal treatments over 200 °C indicates that offal products are among the types of meat that produce lowest exposure to HA.

1. Introduction

Since early times people cooked meat to increase its safety and palatability, however heat-processing temperatures favour reactions between compounds inherent in meat and fish yielding genotoxic substances (Jägerstad & Skog, 2005). HAs are mutagens produced at ppb levels when meat or fish are thermally processed. To date, more than 20 HAs have been isolated and characterised from heated protein-rich foods and model systems (Skog, Johansson, & Jägerstad, 1998). HAs have been classified into two main families, aminoimidazoazaarenes and aminocarbolines. Aminoimidazoazaarenes, such as DMIP, IQ, MeIQ, MeIQx, 4,8-DiMeIQx and PhIP, are formed at normal cooking temperatures through aldol condensation of pyridines and pyrazines, resulted in the Maillard reaction between amino acids and sugars, Strecker aldehydes and creatinine. Some aminocarbolines, such as AαC, MeAαC, Trp-P-1 and Trp-P-2, are produced by pyrolysis of amino acids and proteins at temperatures above 300 °C (Jägerstad, Skog, Grivas, & Olsson, 1991; Milic, Djilas, & Canadanovicbrunet, 1993; Pearson, Chen, Gray, & Aust, 1992).

Although the exposure to HAs through diet is lower than the doses used in carcinogenic assays with animals, the daily exposure over a lifetime could produce cancer. Several epidemiological studies have shown positive correlations between intakes of HAs and increased risk of different types of human cancer (Destefani, Ronco, Mendilaharsu, & Deneopellegrini, 1998; Destefani, Ronco, Mendilaharsu, Guidobono, & Deneopellegrini, 1997), whereas other studies have not found such correlation (Augustsson, Skog, Jägerstad, Dickman, & Steineck, 1999; Gunter et al., 2005). The possible involvement of HAs in human cancer has recently been discussed (Totsuka, Nishigaki, Sugimura, & Wakabayashi, 2006). IARC classified several HAs as *probable* and *possible human carcinogens* and recommended a decrease in their intake (International Agency for Research on International Agency for Research on International Agency for Research on Cancer, 1993).

In order to estimate the intakes and risks to human health is important to quantify HAs in different meat products prepared in different ways. The variety and amounts of HAs are determined by cooking method (time, temperature, heat transfer, ingredients) and the composition of meat/fish (Jägerstad, Skog, Arvidsson, & Solyakov, 1998). All these suggest that a diminution of HAs intake is possible by adopting minor changes in recipes when preparing different meat dishes such as decreasing cooking time and temperature, not including meat drippings in the dish, adding water-holding ingredients to lower the transport of water soluble precursors to the food surface or using different marinades (Skog & Jägerstad, 2006).

The objective of this study was to determine for the first time the occurrence of HAs in offal products with the aim to identify new potential sources of HAs and how the levels and type of HAs could be affected by the addition of cooking ingredients normally used in cooking procedures in Spain. In addition, the present study seeks to find food items with low amount of HAs, in order to be able to propose types of meat that could be used to prepare healthier meat derived products. Earlier works revealed low mutagenicity levels in thermally processed offal products (Bjeldanes et al., 1982; Laser Reuterswärd, Skog, & Jägerstad, 1987), therefore it was still necessary to determine the type and level of HAs in these foodstuffs.

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2. Materials and methods

2.1. Materials

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-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (4,8-DiMeIQx), 2-amino-3,4,7,8-tetramethylimidazo[4,5-*f*]quinoxaline (4,7,8-TriMeIQx), 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), 3-amino-1,4-dimethyl-5H-pyrido[4,3-*b*]indole (Trp-P-1), 3-amino-1-methyl-5H-pyrido[4,3-*b*]indole (Trp-P-2), 2-amino-9H-pyrido[2,3-*b*]indole (AαC), 2-amino-3-methyl-9H-pyrido[2,3-*b*]indole (MeAαC) were purchased from Toronto Research Chemicals (Toronto, Canada), and 1-methyl-9H-pyrido[3,4-*b*]indole (harman) and 9H-pyrido[3,4-*b*]indole (norharman) were purchased from Sigma (Missouri, USA). The chemical purity of the reference compounds was higher than 99%. 4,7,8-TriMeIQx was used as internal standard. Stock standard solutions of each amine in methanol were prepared at 100 μg g^{-1} and used for further dilutions.

All solvents used were of HPLC grade and purchased from Merck (Darmstadt, Germany). Water was purified in a Milli Q Simplicity 185 system (Millipore, Bedford, MA, USA). Extrelut NT20 extraction cartridges were provided by Merck (Darmstad, Germany), and Isolute HM-N™ refill material was obtained from IST (Hengoed, UK). Bond Elut propylsulfonic acid PRS (500 mg) and octadecylsilane C_{18} (100 mg) cartridges, coupling pieces and stopcocks were from Varian (Harbor City, USA).

2.2. Instrumentation

Temperature was measured with type K insulated-wire probes and monitored with Normadics TC6 software from Cole-Parmer (Vernon Hills, IL, USA).

A common blender was used for grinding sample crusts. An Ultra-Turrax® T25 basic (IKA, Staufen, Germany) was used to homogenise ground sample crust mixed with 1 M sodium hydroxide solution. Samples were extracted with SPE vacuum manifold from Supelco (Supelco, Gland, Switzerland).

An Agilent 1100 HPLC (Agilent Technologies, Waldbronn, Germany) equipped with an autosampler and an API 3000™ triple quadrupole mass spectrometer with a turbo ion spray source operating in positive mode (Perkin-Elmer Sciex, Concord, ON, Canada) was used with a 5 μm particle size C_8 Symmetry LC column (Waters, Milford, MA, USA), 150 mm × 2.1 mm I.D.

2.3. Food samples

Raw beef liver, lamb kidney and beef tongue as well as the ingredients including salt, pepper, bay-leaf, saffron, garlic, onion, almonds, lemons, pomegranates, lard, olive oil, sherry, white and red wine were purchased from a local supermarket in Barcelona. The skin from beef tongue was peeled off and visible fat was trimmed from lamb kidney. Beef liver was cut into cubes of approximately 3 cm, lamb kidney was sliced crosswise (0.8 cm thickness) and beef tongue was sliced into 1.5 cm thick fillets. The cooking methods used were frying and stir frying. According to the Eurocode descriptor system (COST Action 99/EUROFOODS), frying implies cooking on heated oil or fat which then becomes an ingredient of the finished product and stir frying denotes frying over high heat by stirring constantly. The heat source was an electric cooker and a Teflon-coated frying pan (270 mm × 270 mm) was used in these experiments. The cooking process began when the temperature in the centre of the pan reached 210–225 °C and kept constant in that range for 5 min. All samples were cooked until lightly browned (4 min total cooking time) adding olive oil to prevent sticking to the metal surface. Cooking methods are described in Table 1, it can be seen that several ingredients were added and additional thermal treatment was carried out. After stir frying, all the samples were drained off using filter paper to remove pan residues and gravies. The crust (2–3 mm) was separated from the moist meat interior, grounded with a food blender, bottled and stored at −18 °C until analysis. Weight loss was measured as the difference between weight of samples before and after frying.

2.4. Extraction of HAs

HAs from offal samples were extracted and purified according to the method developed and validated by Toribio, Busquets, Puignou, and Galceran (2007) and Toribio, Puignou, and Galceran (1999). The samples were removed from storage (−18 °C) and allowed to equilibrate at room temperature. First, 20–40 g of 1 M NaOH (depending on samples water content) was added to 15 g of meat sample and the mixture was homogenised using an

Table 1
Description of food processing

Meat	Cooking recipes[a,b]	Cooking method	Cooking loss (%)
Liver (1)	Liver (256 g) was stir fried in olive oil (30 ml) until lightly browned and removed (4 min). Salt (1 g); peppercorns (4); saffron (1 g); fried garlic (2 g) and fried skinned almonds (6) were crushed, mixed with water (15 ml) and added to the pan with the stir fried liver. White wine (50 ml) and a bay-leaf were added and liver was cooked in the sauce (20 min)	Stir frying	31.0
Liver (2)	Liver (285 g) was stir fried in olive oil (33 ml) until browned (4 min) and removed. Flour (15 g), red wine (60 ml), lemon juice (8 ml), salt (1 g) and pepper (0.3 g) were stirred in. Liver was returned to the pan and cooked until the sauce was thickened (10 min)	Stir frying	23.2
Liver[c]	Liver (214 g) was stir fried in olive oil (25 ml) until lightly browned and removed (4 min)	Stir frying	32.1
Kidney	Kidneys (197 g) were stir fried in olive oil (23 ml) until lightly browned (4 min) and removed. A bay-leaf, flour (6 g), pepper (0.3 g) and sherry (40 ml) were added to fried onion (25 g) and garlic (2 g) in the pan and cooked (10 min). Kidneys were simmered in the sauce (5 min)	Stir frying	49.2
Kidney[c]	Kidneys (211 g) were stir fried in olive oil (25 ml) until lightly browned (4 min) and removed	Stir frying	49.4
Tongue	Tongue (412 g) was boiled (25 min) and the skin from the boiled tongue was removed. Peeled tongue was fried in lard (50 g) with onion (50 g) until lightly browned (4 min). Pomegranate (50 g), salt (1 g), pepper (0.3 g) and sherry (50 ml) were added to the pan and tongue was simmered (1 h)	Frying	26.6
Tongue[c]	Tongue (186 g) was boiled (25 min) and the skin from the boiled tongue was removed. Peeled tongue was fried in lard (23 g) until lightly browned (4 min)	Frying	39.7

[a] Cooking temperature ranged between 210 and 225 °C.
[b] Amount of ingredients and cooking time are given in brackets.
[c] Offal products cooked without ingredients.

Ultra-Turrax T25 basic. Then, an amount corresponding to 3 g of animal visceral was thoroughly mixed with 13 g of diatomaceous earth. The mixture was transferred to an empty Extrelut column coupled to a Bond Elut PRS cartridge, which was previously preconditioned with 5 ml 0.1 M HCl, 10 ml of water and 5 ml MeOH. The analytes were extracted from diatomaceous earth and eluted to the PRS cartridge using 75 ml ethyl acetate. The PRS cartridge was vacuum-dried and rinsed successively with 15 ml of methanol/water (4:6, v/v) and 2 ml water. Next, the PRS cartridge was coupled to a Bond Elut C_{18} cartridge, which was previously preconditioned with 5 ml of MeOH and 5 ml of water, and HAs were desorbed with 20 ml of 0.5 M ammonium acetate adjusted at pH 8.5 with ammonia solution. Finally, the C_{18} cartridge was rinsed with 5 ml of water, dried under slight vacuum, and HAs were eluted to a vial using 0.8 ml of methanol/ammonia (9:1, v/v). The solvent was gently evaporated under a stream of nitrogen and the residue was reconstituted in 0.1 ml of 4,7,8-TriMeIQx in methanol.

2.5. Identification and quantification of HAs

Quantification and recoveries calculation were performed by the standard addition method, which comprised two unspiked samples and two spiked samples at different level (50% and 100%) for the beef items and four spiking levels (50%, 100%, 150% and 200%) for the lamb items. Lamb samples was quantified by external calibration curves for each amine were constructed using five calibration standard solution with concentrations ranging from 0.3 ng g^{-1} to 1 μg g^{-1}. Recoveries were calculated from the slope of the linear regression obtained between the added analyte concentration and the measured analyte concentration.

Table 2
MRM parameters used with the triple quadrupole instrument

HAs	Precursor ion $[M^+H]^+ m/z$	Quantification product ion (m/z)	Confirmation product ion (m/z)	Collision offset voltage (V)
DMIP	163	148	147	37
IQ	199	184	157	39
MeIQ	213	198	–	38
MeIQx	214	199	173	38
4,8-DiMeIQx	228	213	187	40
4,7,8-TriMeIQx	242	227	201	38
Norharman	169	115	–	49
Harman	183	115	168	49
PhIP	225	210	–	43
Trp-P-1	212	195	168	36
Trp-P-2	198	181	154	35
AαC	184	167	140	38
MeAαC	198	181	154	35

[a]Interchannel time delay: 5 ms.
[b]Dwell time: 150 ms.

Optimum separation of HAs was achieved with a binary mobile phase at a flow rate of 0.3 ml·min^{-1}. Solvent A: acetonitrile; solvent B: 30 mM formic acid-ammonium formate buffer at pH 3.7. The gradient elution program was: 0–1 min, 5% A; 1–15 min, 5–30%; 15–18 min, 30–60% A; 18–30 min, 60% A; 10 min equilibration of the column. The sample volume injected was 5 μl. Data acquisition was performed in multiple reaction monitoring (MRM). For quantification, 13 MRM transition were monitored simultaneously. The precursor ions selected were the protonated molecular ions $[M+H]^+$ The most abundant product ions were monitored for HAs quantification, and in a second acquisition, the second most abundant product ions were monitored to confirm the identity of the analytes. In Table 2, the MRM transitions used for quantification and confirmation are given. Briefly, working parameters were: electrospray voltage, 2.5 kV; nebuliser gas, 11 a.u.; curtain gas, 14 a.u.; turbo ionspray gas flow rate, 7000 a.u.; turbo ionspray gas temperature, 450 °C; declustering potential, 30 V. In Table 2 MRM parameters are given. Data acquisition was carried out by Analyst 1.4.2 software.

3. Results and discussion

In this study four offal dishes were cooked and analysed for twelve HAs. The meats were cooked in two ways. The first one, stir fried or fried with olive oil and without using other additives. The second one processed according to the described cooking recipes in Table 1. The results of HAs determination in offal products are presented in Table 3, where it can be observed that norharman and harman were the HAs most frequently detected in the offal dishes, with or without using cooking additives, in amounts up to 8.87 ng g^{-1} and 3.10 ng g^{-1}, respectively. 4,8-DiMeIQx was only detected in kidney and tongue control samples, at levels ranging from <0.01 to 0.02 ng g^{-1}, and DMIP and MeIQx were detected up to 0.25 ng g^{-1} in the same samples. PhIP was also detected in low amounts, up to 0.12 ng g^{-1}, in the offal samples. IQ, Trp-P-1, Trp-P-2, AαC and MeAαC were not detected in any of the cooked tissues. Limits of detection and extraction efficiencies are given in Table 4. Recovery rates estimated for DMIP, IQ, norharman, harman and MeAαC were higher than 70%. These amines were extracted more efficiently from the offal products than from the beef samples studied in a previous work following the same clean-up method except that dichloromethane was used instead of ethyl acetate as extraction solvent (Toribio, Busquets, Puignou, & Galceran, 2007). The improvement in the extraction can be due to the different composition of both the meat and the extraction solvent. The recovery rates of the other HAs studied ranged between 20% and 53%.

To illustrate the results, the HAs detected in the control kidney sample are shown in Fig. 1. Notice that good sensitivity was achieved in the determination, even though 13 MRM transitions were acquired at the same time.

Table 3
Heterocyclic amines content in cooked offal samples (ng $g^{-1} \pm s$[a])

Sample	DMIP	IQ	MeIQx	MeIQ	Norharman	Harman	PhIP	4,8-DiMeIQx	Trp-P-1	Trp-P-2	AαC	MeαAC
Beef liver (1)	nd	nd	nd	nd	8.87 ± 1.84	3.10 ± 1.13	nd	nd	nd	nd	nd	nd
Beef liver (2)	nd	nd	nd	nd	1.87 ± 0.30	1.83 ± 0.30	0.10 ± 0.32	nd	nd	nd	nd	nd
Beef liver[b]	nd	nd	nd	nd	1.93 ± 0.43	0.30 ± 0.31	nd	nd	nd	nd	nd	nd
Lamb kidney	nd	nd	nd	nd	0.45 ± 0.13	0.32 ± 0.17	0.12 ± 0.04	nd	nd	nd	nd	nd
Lamb kidney[b]	0.25 ± 0.18	nd	0.08 ± 0.04	nd	0.50 ± 0.10	0.55 ± 0.24	0.11 ± 0.08	<0.01[c]	nd	nd	nd	nd
Beef tongue	nd	nd	<0.04	<0.1	0.83 ± 0.41	0.33 ± 0.20	<0.03	nd	nd	nd	nd	nd
Beef tongue[b]	<0.1	nd	0.21 ± 0.45	nd	0.61 ± 0.38	0.20 ± 0.17	0.02 ± 0.03	0.02 ± 0.04	nd	nd	nd	nd

nd: Not detected.
[a] Standard deviation obtained from addition standard calibration curve.
[b] Control offal products cooked without additives.
[c] Amount detected below the limit of quantification (signal-to-noise ratio of 10).

Table 4
Estimated recovery rates and limits of detection of HAs in cooked offal samples

Sample	DMIP		IQ		MeIQx		MeIQ		Norharman		Harman		PhIP		4,8-DiMeIQx		Trp-P-1		Trp-P-2		AαC		MeAαC	
	R[a]	LOD[c]	R	LOD	R	LOD	R	LOD	R	LOD	R	LOD	R	LOD	R	LOD	R	LOD	R.	LOD	R	LOD	R	LOD
Beef liver (1)	79	0.05	72	0.04	44	0.03	20	0.03	89	0.03	90	0.02	40	0.01	35	0.02	26	0.03	34	0.02	20	0.02	84	0.03
Beef liver (2)	80	0.04	75	0.04	48	0.03	22	0.03	90	0.03	89	0.02	43	0.01	40	0.02	29	0.02	32	0.02	20	0.02	84	0.03
Beef liver[b]	83	0.04	78	0.03	46	0.03	21	0.03	92	0.03	93	0.02	44	0.01	39	0.02	29	0.02	37	0.02	21	0.02	85	0.03
Lamb kidney	82	0.04	76	0.04	49	0.02	20	0.04	92	0.03	93	0.01	37	0.01	39	0.004	28	0.02	29	0.03	20	0.01	89	0.02
Lamb kidney[b]	85	0.04	79	0.04	53	0.01	23	0.03	91	0.03	94	0.01	43	0.01	44	0.004	27	0.03	33	0.02	22	0.01	88	0.02
Beef tongue	80	0.03	77	0.04	51	0.01	20	0.04	89	0.03	95	0.02	43	0.01	44	0.01	31	0.02	25	0.03	20	0.02	87	0.02
Beef tongue[b]	79	0.03	80	0.03	50	0.02	20	0.03	90	0.03	92	0.02	47	0.01	48	0.01	33	0.02	29	0.02	20	0.02	89	0.02

[a] R: Recovery (%).
[b] Control offal products cooked without additives.
[c] LOD: Limit of detection (ng g^{-1}) estimated at a signal-to-noise ratio of 3.

In agreement with the results found, IQ, MeIQ, Trp-P-1, Trp-P-2, AαC and MeAαC have not been very often detected in cooked meats, this fact may be due to higher temperatures are necessary for their formation (Arvidsson, Vanboekel, Skog, & Jägerstad, 1997; Jägerstad et al., 1998), though they have been detected at low levels (<1 ng g^{-1}) after thermally processing meats at temperatures similar than those reached in the present study (Busquets, Bordas, Toribio, Puignou, & Galceran, 2004; Ristic, Cichna, & Sontag, 2004). On the other hand, DMIP, MeIQx, 4,8-DiMeIQx are generally formed above 0.1 ng g^{-1} at cooking temperatures around 200 °C (Busquets et al., 2004; Skog et al., 1998) whereas, the amount of these HAs found in the offal products were at least ten fold lower. Nevertheless, the cooking conditions applied in the present study are difficult to compare with previous published works. However, the levels of PhIP quantified in the cooked offal products were very low compared to the amounts found in the analysed food items reported in the literature, that use to range 0.2–15 ng g^{-1}, being the highest amounts in poultry meat generally, and the lowest amounts in hamburgers, sausages, and kebabs, industrially prepared or from fast food outlets and restaurants (Borgen & Skog,

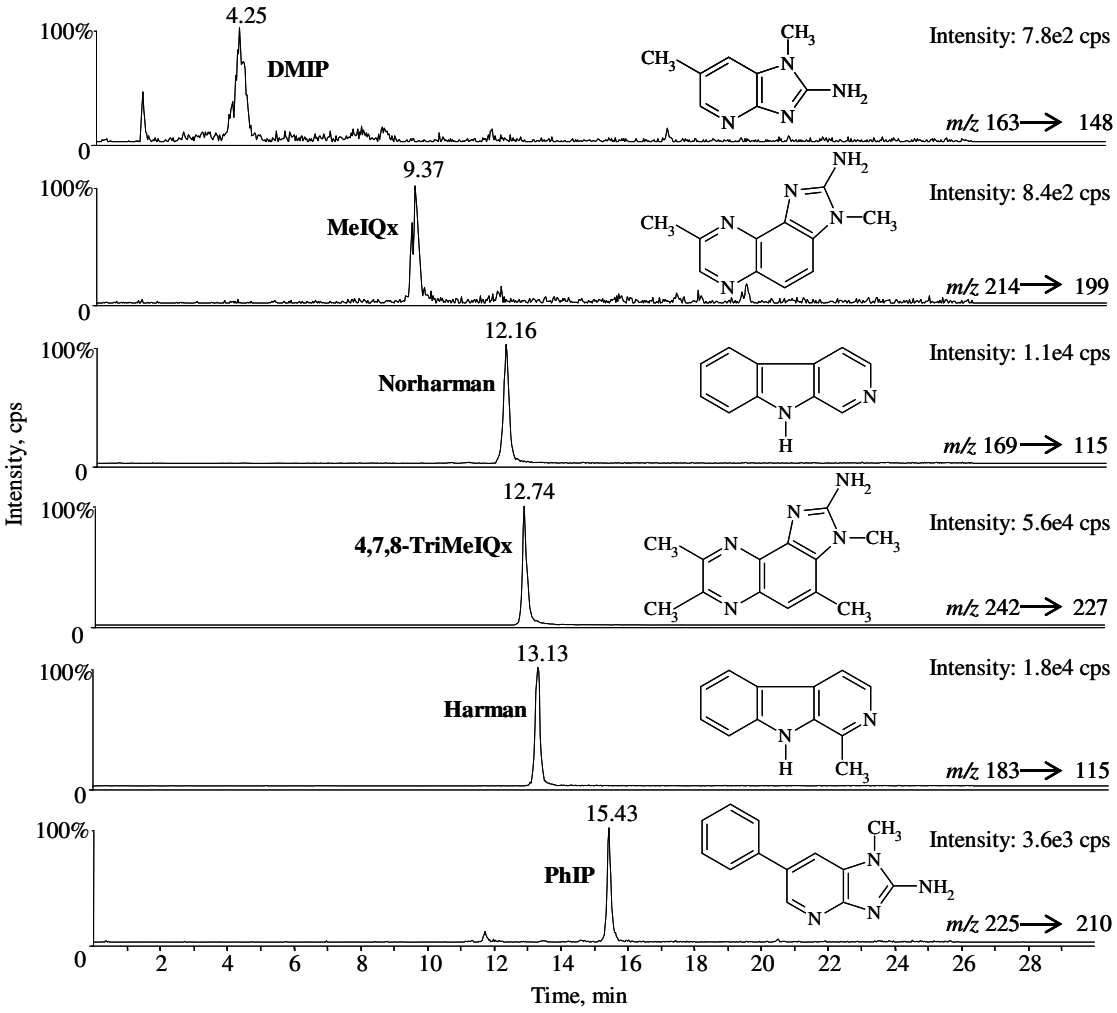

Fig. 1. Chromatograms of HAs detected in lamb kidney control sample determined by liquid chromatography–tandem mass spectrometry in MRM acquisition mode. Transitions between precursor and product ions are indicated in each window.

2004; Knize et al., 1995). Indeed, PhIP has been pointed out as one of the HA with major contribution to the daily intake of HAs (Augustsson, Skog, Jägerstad, & Steineck, 1997; Busquets et al., 2004; Keating & Bogen, 2004; Kobayashi, Hanaoka, Nishioka, Kataoka, & Tsugane, 2002) and the detection of such low levels of this ubiquitous HA in thermally processed proteinaceous food is a peculiarity.

Generally, as compared to other meat products, low levels of HAs in all offal samples were formed. This is in agreement with the results obtained by Laser Reuterswärd et al. (1987) who detected low levels of mutagenicity in offal fried patties and associated the findings with the low content of creatine and creatinine.

The levels of HAs are affected by the heat transference during the cooking process (Skog & Jägerstad, 2006). In the present work, kidney and liver were processed by stir frying and low levels of HAs were formed. In this cooking method, meat is less in contact with the heating surface because of the frequently stirring, which affects the heat transfer. Salmon et al. (2000) demonstrated that reduced amounts of HAs were formed by frequently turning the meat when cooking. In contrast, the tongue was cooked by boiling and frying, and even though frying processes provide more heat transference than stir frying, the amounts of HAs generated were also low (<1 ng g^{-1}) which indicates that the meat composition influenced the level of formation.

The offal dishes cooked using food additives contained lower levels of most of the analysed aminoimidazoazaarenes as compared to the corresponding control samples (Table 3) whereas aminocarbolines were not detected in any of the samples with the exception of norharman and harman, which increased their amount in one of the liver samples with the additional cooking time. Thus, the additional heating from 10 min and up to 1 h did not produce a pronounced increase on the amounts of HAs. This fact may indicate that some of the ingredients used during cooking might have inhibition properties on the formation of HAs, however in each recipe several ingredients were used, therefore it was difficult to find out any specific conclusion relating to the effect of each additive. The radical scavenging properties of some of the additives could have interfered in the formation of HAs. For instance several natural ingredients rosemary, thyme, sage, brine, garlic (Murkovic, Steinberger, & Pfannhauser, 1998); wine (Busquets, Puignou, Galceran, & Skog, 2006; Busquets, Puignou, Galceran, Wakabayashi, & Skog, 2007) or marinades involving a mixture of ingredients (Salmon, Knize, & Felton, 1997) reduced the content of some HAs during cooking processes.

4. Conclusions

HAs have been analysed in several offal products such as beef liver, lamb kidney and beef tongue for the first time to our knowledge. Norharman and harman were the most frequently HAs found in all the samples in levels ranging from 0.20 to 8.87 ng g^{-1}, whereas DMIP ($\leqslant$0.25 ng g^{-1}), MeIQx ($\leqslant$0.21 ng g^{-1}), MeIQ (<0.1 ng g^{-1}), PhIP ($\leqslant$0.12 ng g^{-1}) and 4,8-DiMeIQx ($\leqslant$0.02 ng g^{-1}), were only detected in few of the studied samples. Other HAs, IQ, Trp-P-1, Trp-P-2, AαC and MeAαC, were not detected in any analysed samples. The levels of HAs found in the analysed cooked offal products are low compared with the levels found in other types of meat. Therefore this type of meat could be proposed for the elaboration of meat derived products with low content of HAs. This study also shows that by using cooking practices that include additives the levels of HAs can be kept low even if prolonged thermal treatments are applied. This data could be used to assess the human intake of HAs in Spain and contributes to the search of simple cooking practices that minimize the risk of exposure to HAs, and thus to improve food quality and safety.

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References

Arvidsson, P., Vanboekel, M. A. J. S., Skog, K., & Jägerstad, M. (1997). Kinetics of formation of polar heterocyclic amines in a meat model system. *Journal of Food Science, 62*, 911–916.

Augustsson, K., Skog, K., Jägerstad, M., Dickman, P. W., & Steineck, G. (1999). Dietary heterocyclic amines and cancer of the colon, rectum, bladder, and kidney – A population-based study. *Lancet*, 703–707.

Augustsson, K., Skog, K., Jägerstad, M., & Steineck, G. (1997). Assessment of the human exposure to heterocyclic amines. *Carcinogenesis, 18*, 1931–1935.

Bjeldanes, L. F., Morris, M. M., Felton, J. S., Healy, S., Stuermer, D., Berry, P., et al. (1982). Mutagens from the cooking of food. III. Survey by Ames/Salmonella test of mutagen formation in secondary sources of cooked dietary protein. *Food and Chemical Toxicology, 20*, 365–369.

Borgen, E., & Skog, K. (2004). Heterocyclic amines in some Swedish cooked foods industrially prepared or from fast food outlets and restaurants. *Molecular Nutrition & Food Research, 48*, 292–298.

Busquets, R., Bordas, M., Toribio, F., Puignou, L., & Galceran, M. T. (2004). Occurrence of heterocyclic amines in several home-cooked meat dishes of the Spanish diet. *Journal of Chromatography B, 802*, 79–86.

Busquets, R., Puignou, L., Galceran, M. T., & Skog, K. (2006). Effect of red wine marinades on the formation of heterocyclic amines in fried chicken breast. *Journal of Agricultural and Food Chemistry, 54*, 8376–8384.

Busquets, R., Puignou, L., Galceran, M. T., Wakabayashi, K., & Skog, K. (2007). Liquid chromatography-tandem mass spectrometry analysis of 2-amino-1-methyl-6-(4-hydroxyphenyl)imidazo[4,5-b]pyridine in cooked meats. *Journal of Agricultural and Food Chemistry, 55*, 9318–9324.

International Agency for Research on Cancer (1993) IARC monographs on the evaluation of carcinogenic risks to humans. *Some natural occurring substances: Food items and constituents. Heterocyclic amines and mycotoxins* (Vol. 56, pp. 163–242). Lyon: International Agency for Research on Cancer.

Destefani, E., Ronco, A., Mendilaharsu, M., & Deneopellegrini, H. (1998). Case-control study on the role of heterocyclic amines in the etiology of upper aerodigestive cancers in Uruguay. *Nutrition and Cancer, 32*, 43–48.

Destefani, E., Ronco, A., Mendilaharsu, M., Guidobono, M., & Deneopellegrini, H. (1997). Meat intake, heterocyclic amines, and risk of breast-cancer – A case-control study in Uruguay. *Cancer Epidemiology, Biomarkers & Prevention, 6*, 573–581.

Gunter, M. J., Probst-Hensch, N. M., Cortessis, V. K., Kulldorff, M., Haile, R. W., & Sinha, R. (2005). Meat intake, cooking-related mutagens and risk of colorectal adenoma in a sigmoidoscopy-based case-control study. *Carcinogenesis, 26*, 637–642.

Jägerstad, M., & Skog, K. (2005). Genotoxicity of heat-processed foods. *Mutation Research, 574*, 156–172.

Jägerstad, M., Skog, K., Arvidsson, P., & Solyakov, A. (1998). Chemistry, formation and occurrence of genotoxic heterocyclic amines identified in model systems and cooked foods. *Zeitschrift für Lebensmitteluntersuchung und Forschung A, 207*, 419–427.

Jägerstad, M., Skog, K., Grivas, S., & Olsson, K. (1991). Formation of heterocyclic amines using model systems. *Mutation Research, Genetic Toxicology Testing, 259*, 219–233.

Keating, G. A., & Bogen, K. T. (2004). Estimates of heterocyclic amine intake in the US population. *Journal of Chromatography B, 802*, 127–133.

Knize, M. G., Sinha, R., Rothman, N., Brown, E. D., Salmon, C. P., Levander, O. A., et al. (1995). Heterocyclic amine content in fast-food meat-products. *Food and Chemical Toxicology, 33*, 545–551.

Kobayashi, M., Hanaoka, T., Nishioka, S., Kataoka, H., & Tsugane, S. (2002). Estimation of dietary HCA intakes in a large-scale population-based prospective study in Japan. *Mutation Research*, 233–241.

Laser Reuterswärd, A., Skog, K., & Jägerstad, M. (1987). Mutagenicity of pan-fried bovine tissues in relation to their content of creatine, creatinine, monosaccharides and free amino acids. *Food and Chemical Toxicology, 25*, 755–762.

Milic, B. L., Djilas, S. M., & Canadanovicbrunet, J. M. (1993). Synthesis of some heterocyclic aminoimidazoazarenes. *Food Chemistry, 46*, 273–276.

Murkovic, M., Steinberger, D., & Pfannhauser, W. (1998). Antioxidant spices reduce the formation of heterocyclic amines in fried meat. *Zeitschrift für Lebensmitteluntersuchung und Forschung A, 207*, 477–480.

Pearson, A. M., Chen, C., Gray, J. I., & Aust, S. D. (1992). Mechanism(s) involved in meat mutagen formation and inhibition. *Free Radical Biology & Medicine, 13*, 161–167.

Ristic, A., Cichna, M., & Sontag, G. (2004). Determination of less polar heterocyclic aromatic amines in standardised beef extracts and cooked meat consumed in

Austria by liquid chromatography and fluorescence detection. *Journal of Chromatography B, 802*, 87–94.

Salmon, C. P., Knize, M. G., & Felton, J. S. (1997). Effects of marinating onheterocyclic amine carcinogen formation in grilled chicken. *Food and Chemical Toxicology, 35*, 433–441.

Salmon, C. P., Knize, M. G., Panteleakos, F. N., Wu, R. W., Nelson, D. O., & Felton, J. S. (2000). Minimization of heterocyclic amines and thermal inactivation of Escherichia coli in fried ground beef. *Journal of the National Cancer Institute, 92*, 1773–1778.

Skog, K., & Jägerstad, M. (2006). Modifying cooking conditions and ingredients to reduce the formation of heterocyclic amines. In K. Skog & J. Alexander (Eds.), *Acrylamide and other hazardous compounds in heat-treated foods* (pp. 407–424). Cambridge: Woodhead Publishing Ltd.

Skog, K., Johansson, M. A. E., & Jägerstad, M. I. (1998). Carcinogenic heterocyclic amines in model systems and cooked foods – A review on formation, occurrence and intake. *Food and Chemical Toxicology, 36*, 879–896.

Toribio, F., Busquets, R., Puignou, L., & Galceran, M. T. (2007). Heterocyclic amines in griddled beef steak analysed using a single extract clean-up procedure. *Food and Chemical Toxicology, 45*, 667–675.

Toribio, F., Puignou, L., & Galceran, M. T. (1999). Evaluation of different cleanup procedures for the analysis of heterocyclic aromatic-amines in a lyophilized meat extract. *Journal of Chromatography A, 836*, 223–233.

Totsuka, Y., Nishigaki, R., Sugimura, T., & Wakabayashi, K. (2006). The possible involvement of mutagenic and carcinogenic heterocyclic amines in human cancer. In K. Skog & J. Alexander (Eds.), *Acrylamide and other hazardous compounds in heat-treated foods* (pp. 296–327). Cambridge: Woodhead Publishing Ltd.