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Effects of precursor composition and water on the formation of heterocyclic amines in meat model systems

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Abstract

Model systems based on pressed meat from ox, pork and chicken were used to study the formation of carcinogenic/mutagenic heterocyclic amines (HAs). The composition of precursors (free amino acids, creatine and glucose) was examined and samples were heated in test-tubes under wet and dry conditions at 175 and 200°C for 30 min. Several HAs were detected, and the formation of DMIP (2-amino-1,6-dimethylimidazo[4,5-*b*]-pyridine), TMIP (2-amino-1,5,6-trimethyl-imidazo[4,5-*b*]-pyridine), IFP (2-amino-1,6-dimethylfuro[3,2-*e*]imidazo[4,5-*b*]-pyridine) and PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-*b*]-pyridine) was found to be favoured by dry heating conditions. Highest amounts of PhIP and IFP were detected in heated meat juice from chicken breast, while more MeIQx (2-amino-3,8-dimethylimidazo[4,5-*f*]-quinoxaline) was found in heated meat juices from roast beef and pork chop. Norharman (9H-pyrido[3,4-*b*]-indole) and Harman (1-methyl-9H-pyrido[3,4-*b*]-indole) were also detected at high levels.

Keywords: Heterocyclic amines; Food mutagens; Model system; Meat juice; Creatine; Amino acids; Glucose; Meat; Chicken; Pork; Beef

1. Introduction

Carcinogenic/mutagenic heterocyclic amines (HAs) are formed in muscle meat at ppb levels during heating at common household cooking temperatures. In 1977, extracts of charcoal-fried fish were found to contain mutagenic substances (Nagao, Honda, Seino, Yahagi & Sugimura, 1977; Sugimura et al., 1977) which were later identified as HAs. Today, more than 20 carcinogenic/mutagenic HAs have been isolated and identified in cooked foods (Felton & Knize, 1990; Sugimura, 1997; Wakabayashi, Nagao, Esumi & Sugimura, 1992). HAs have been found to be multi-site carcinogens in long-term studies in rats, mice and non-human primates (Adamson et al., 1990; Kato, Migita, Ohgaki, Sato, Takayama & Sugimura, 1989; Ohgaki et al., 1984). Furthermore, it has been shown, in animal studies, that HAs pass into breast milk (Brittebo, Karlsson, Skog & Jägerstad, 1994; Ghoshal & Snyderwine, 1993), and that HAs are strongly carcinogenic in neonatal mice exposed to 5000–10,000 times lower doses than are usually used in animal studies (Dooley, Stavenuiter, Westra & Kadlubar, 1988). The International Agency for Research on Cancer regards some of the HAs, tested to date, as possible human carcinogens (class 2B) and one as a probable human carcinogen (class 2A; IARC, 1993). Human cells metabolise HAs and form DNA adducts (Boobis et al., 1995; Lin, Lang & Kadlubar, 1995; Turteltaub et al., 1997). Differences in the enzymatic systems that metabolise HAs may suggest a carcinogenic effect of HAs in humans who are genetically susceptible and/or are highly exposed to them (Felton, Malfatti, Knize, Salmon, Hopmans & Wu, 1997; Voskuil, 1999; Sugimura, 2000).

To increase our knowledge on the human risk from exposure to HAs, data are being collected on the type and amounts of HAs in common foods and food consumption patterns. It has earlier been shown that, when fried, different sorts of meat form different types and amounts of HAs (for review see Knize, Dolbeare, Cunningham & Felton, 1995; Knize et al., 1998; Skog, Johansson & Jägerstad, 1998; Skog, Steineck, Augustsson & Jägerstad, 1995). Thus there is a need to elucidate precursors and conditions for HA formation. Factors that influence the formation of HAs are the cooking conditions, the amounts of different precursors, e.g. creatine, sugars and free amino acids, present in the meat, and the presence of compounds with enhancing or inhibiting effects. Model systems are useful tools for

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Nomenclature

IQ	2-amino-3-methylimidazo[4,5-*f*]-quinoline, CAS no: 76180-96-6
IQx	2-amino-3-methylimidazo[4,5-*f*]-quinoxaline, CAS no: 108354-47-8
MeIQ	2-amino-3,4-dimethylimidazo[4,5-*f*]-quinoline, CAS no: 77094-11-2
MeIQx	2-amino-3,8-dimethylimidazo[4,5-*f*]-quinoxaline, CAS no: 77500-04-0
7,8-DiMeIQx	2-amino-3,7,8-trimethylimidazo[4,5-*f*]-quinoxaline, CAS no: 92180-79-5
4,8-DiMeIQx	2-amino-3,4,8-trimethylimidazo[4,5-*f*]-quinoxaline, CAS no: 95896-78-9
PhIP	2-amino-1-methyl-6-phenylimidazo-[4,5-*b*]pyridine, CAS no: 105650-23-5
DMIP	2-amino-1,6-dimethylimidazo[4,5-*b*]-pyridine, CAS no: 105650-23-5
TMIP	2-amino-1,5,6-trimethylimidazo[4,5-*b*]-pyridine
IFP	2-amino-1,6-dimethylfuro[3,2-*e*]-imidazo[4,5-*b*]-pyridine
Norharman	9H-pyrido[3,4-*b*]indole, CAS no: 244-63-3
Harman	1-methyl-9H-pyrido[3,4-*b*]indole, CAS no: 486-84-0
Trp-P-2	3-amino-1-methyl-5H-pyrido[4,3-*b*]-indole, CAS no: 62450-10-3
Trp-P-1	3-amino-1,4-dimethyl-5H-pyrido-[4,3-*b*]indole, CAS no: 62450-06-0
EtAc	Ethyl acetate
DCM	Dichloromethane
gdm	gram dry matter
PRS	Propysulfonic Acid Silica
Glu-P-2	2-aminodipyrido[1,2-*a*:3′,2′-*d*]imidazole

studying the influence of different physical and chemical parameters on the formation of HAs (Arvidsson, van Boekel, Skog & Jägerstad, 1997; Pais, Salmon, Knize & Felton, 1999).

The main objective of this study was to examine the effects of precursor composition and water on the formation of HAs in meat model systems, based on muscles with characteristic precursors. Freeze-dried meat juice from ox, pork and chicken were heated under wet and dry conditions to simulate reactions that occur at the meat surface and in the pan residue.

2. Materials and methods

2.1. Chemicals

Solvents and chemicals were of HPLC or analytical grade. Water was passed through a Milli-Q water purification system (Millipore, Bedford, MA, USA). The following HAs were used as reference compounds: Glu-P-1 (2-amino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazole), Glu-P-2 (2-aminodipyrido[1,2-*a*3:′,2′-*d*]imidazole), DMIP (2-amino-1,6-dimethyl-imidazo[4,5-*b*]pyridine), PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine), IQ (2-amino-3-methylimidazo[4,5-*f*]quinoline), MeIQ (2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline), IQx (2-amino-3-methylimidazo[4,5-*f*]quinoxaline), MeIQx (2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline), 4,8-DiMeIQx (2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline), 7,8-DiMeIQx (2-amino-3,7,8-trimethylimidazo[4,5-*f*]quinoxaline), Harman (1-methyl-9H-pyrido[3,4-*b*]indole), Trp-P-1 (3-amino-1,4-dimethyl-5H-pyrido[4,3-*b*]indole) and Trp-P-2 (3-amino-1-methyl-5H-pyrido[4,3-*b*]indole), and were purchased from Toronto Research Chemicals (Toronto, Canada). Norharman (9H-pyrido[3,4-*b*]indole) was purchased from Aldrich (Steinhem, Germany). The chemical purity of the synthetic references was higher than 99%, according to the manufacturers. A mixture of the different HAs (2 ng of each compound/μl) was used as a spiking mixture. TMIP (2-amino-1,5,6-trimethylimidazo[4,5-*b*]pyridine) was bought from the NCI Chemical Carcinogen Reference Standard Repository (Kansas City, MO, USA). IFP (2-amino-1,6-dimethylfuro[3,2-*e*]imidazo[4,5-*b*]pyridine) was kindly provided by Mark G. Knize, Lawrence Livermore National Laboratories, CA, USA. The structures of some of the compounds are shown in Fig. 1. Materials for solid-phase extraction were diatomaceous earth (Isolute), obtained from Sorbent AB (Västra Frölunda, Sweden) and PRS and C_{18} columns (Varian) from Scantech Lab (Partille, Sweden).

2.2. Model systems

Roast beef, pork chops and chicken breasts were purchased in a local store. Meat juice was pressed out of the meat as earlier described (Arvidsson, van Boekel, Skog & Jägerstad, 1999). The yields of fresh meat juice from the roast beef, pork chop and chicken breast were 13, 17 and 12%, respectively. The pieces of meat, now partly depleted of meat juice (here called pressed meat), and the meat juice were freeze-dried and stored at −18°C until used for experiments.

Meat juice was heated in wet and dry model systems (Skog, Solyakov & Jägerstad, 2000) with the aim of mimicking conditions in the crust and pan residue, respectively. In brief, quartz test-tubes containing freeze-dried meat juice were heated in an oil bath at 175 or 200°C for 30 min (dry heating). For wet heating experiments, distilled water was added to the test tubes before heating. Some model experiments were performed with the pressed meat. All experiments were repeated at least once and, in general, performed in duplicate.

2.3. Extraction of HAs

Samples were extracted and purified according to the solid-phase extraction method of Gross and Grüter (1992) with slight modifications (Fay, Ali & Gross, 1997; Knize et al., 1995; Pais et al., 1999). Briefly, samples were dissolved in 1M NaOH and mixed with diatomaceous earth (Isolute), and then transferred to empty columns. EtAc (ethyl acetate) was used as the extraction solvent instead of dichloromethane (DCM). The eluate was passed through PRS columns and C_{18} columns. The method yielded two fractions (polar and less polar HAs), which were evaporated and the residues were then dissolved in 100 μl MeOH. Extraction recovery rates for the different HAs in the model system were determined by the addition of 100 μl spiking mixture to one sample extracted in parallel with two unspiked samples. Samples heated at 175°C were subjected to additional clean-up (Solyakov, Skog & Jägerstad, 1999).

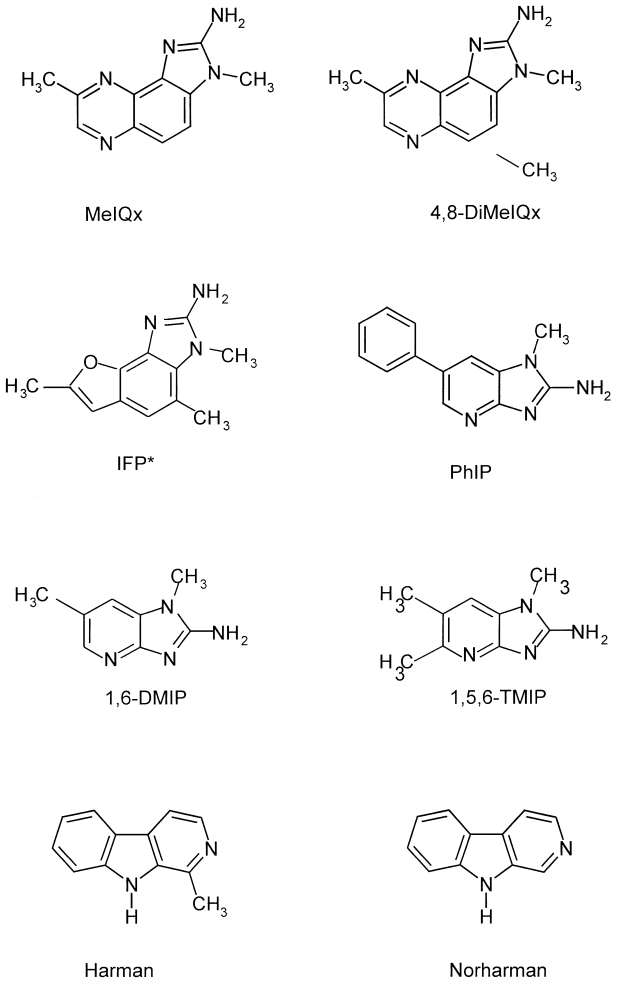

Fig. 1. Structures and trivial names of some HAs found in cooked meat and model systems. * Tentative structure.

2.4. Identification and quantification of HAs

HAs were separated using reverse-phase HPLC (Gross, Grüter & Heyland, 1992) with minor modifications as previously described (Johansson, Skog & Jägerstad, 1993). The column (ODS 80^{TM} TosoHaas, 250×4.6 mm i.d., 5 μ, Labkemi, Lund, Sweden) was eluted with acetonitrile and 0.01 M triethyl amine (pH adjusted to 3.6 with acetic acid). The flow rate was 1 ml/min and the injection volume was 90 μl. Chromatograms and spectra were obtained using a photodiode array UV detector (Varian 9065, Polychrome), and a fluorescence detector (Varian 9070). Peaks were identified and quantified using retention times and the spectra from reference samples of known concentrations, run under the same conditions. The presence of HAs in samples heated at 175°C were confirmed using LC/MS (method to be described elsewhere).

2.5. Chemical analysis

The freeze-dried pressed meat juices were analysed for HA precursors as described by Arvidsson et al. (1997): free amino acid analysis with ion-exchange chromatography, and creatin/in/e and glucose with enzymatic methods.

3. Results and discussion

3.1. Amino acids and HAs

Three HAs that are sparsely reported in the literature, DMIP, TMIP and IFP (Felton, Pais, Salmon & Knize, 1998; Pais et al., 1999; Pais, Tanga, Salmon & Knize, 2000), were detected in several samples. Fig. 2 shows an HPLC chromatogram at 316 nm from dry-heated pressed meat from chicken breast, where peaks corresponding to DMIP, TMIP and IFP are indicated by arrows. The identities of the compounds were confirmed by the retention times, and the sample and library UV absorbance spectra, which are also shown in the figure.

HAs were detected in all heated samples studied and the amounts and species varied between the samples and the heating conditions. In this respect, it is interesting to discuss the yield of HAs in relation to the content of free amino acids, creatine and glucose, which have previously been shown to produce HAs when heated in model systems (review, Skog et al., 1998). The different

meat-derived samples were analysed for these precursors and the contents and proportions of these substances varied greatly in the unheated samples, as shown in Table 1. The 20 common amino acids and taurine were analysed. An interesting observation is that the content of *creatine* was about half that of free amino acids in meat juice from chicken breast, while the opposite was found for meat juice from pork chop and roast beef. Meat juice from beef has previously been shown to have a higher content of creatine than free amino acids (Arvidsson et al., 1999). Furthermore, the total amount of *free amino acids* in chicken breast was about twice as high as in meat juice from pork chop and roast beef. The amount of *glucose* was low in meat juice from chicken breast (3.9 μmol/gdm), and about 4 and 16 times higher in meat juice from pork chop and roast beef. Differences in the amounts of free amino acids, creatine and glucose originate from the natural variation between animal species and between muscles from different parts of the body (Karlsson, 1993). Muscles consist of three main types of fibres (cell types) at different ratios, which can be described using contractile and metabolic characteristics: slow-twitch oxidative (type I), fast-twitch oxidative-glycolytic (type IIA) and fast-twitch glycolytic (type IIB; Brook & Kaiser, 1970). The three cuts of meat used in this study consisted mainly of one or two of the major fibre types. Pork chops and roast beef come from one single muscle, *M. longissimus dorsi*, which consists of about 90% type IIB fibres and less than 10% type I fibres (Karlsson, 1993). Chicken breast is made up of two muscles, *M. pectoralis superficalis* and *M. pectoralis profundus*, which consist mainly of type IIA and type IIB fibres.

In the first set of experiments, meat juice and pressed meat from pork chop and chicken breast were heated at 200°C for 30 min. The amounts of HAs detected in this model system are summarised in Table 2. It is clearly seen that the formation of, for example, PhIP and DMIP is favoured by dry heating conditions, while the opposite is found for MeIQx. Interestingly, the amount of

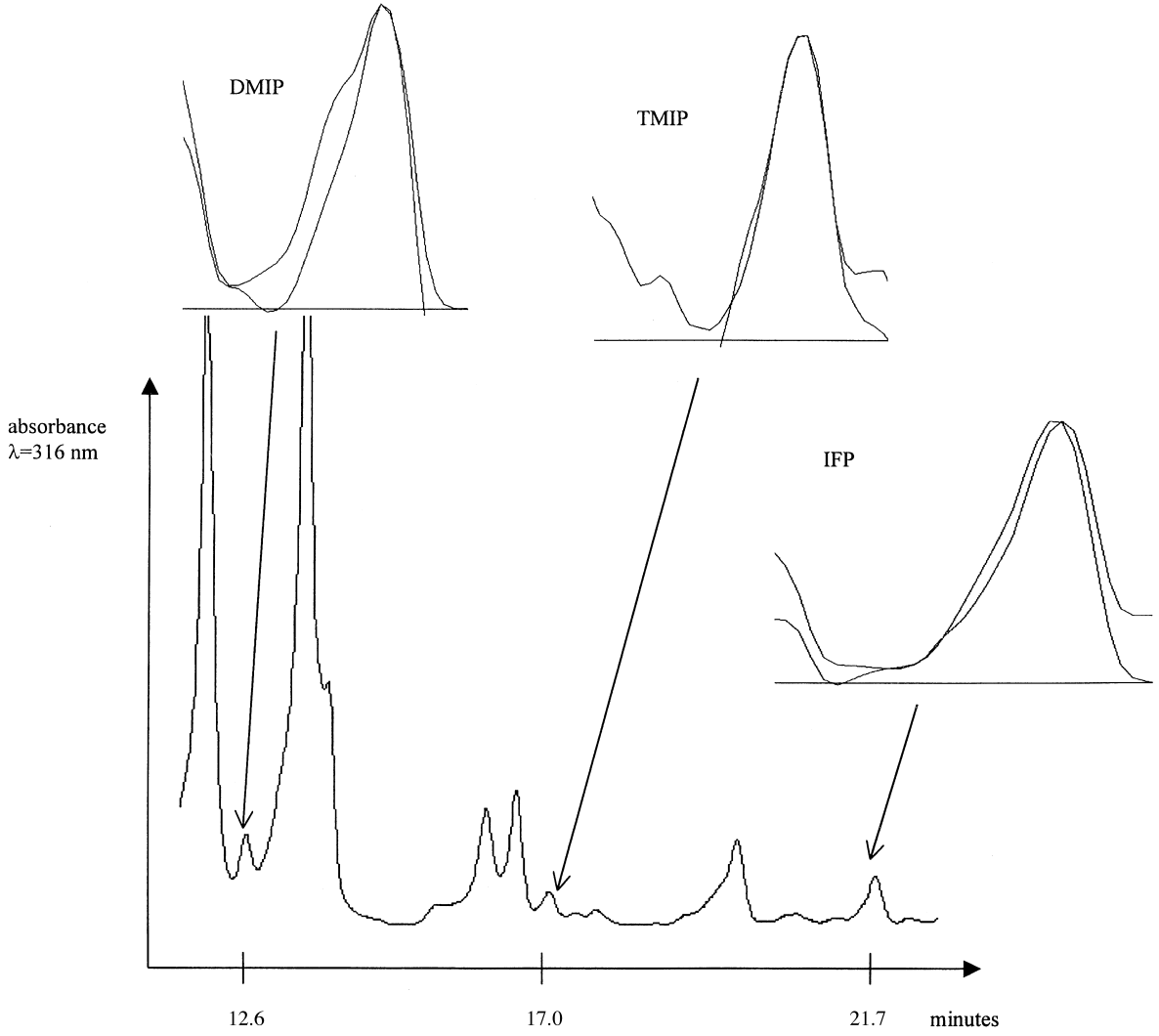

Fig. 2. HPLC chromatogram (316 nm) of a dry-heated sample of pressed chicken breast. Spectra of the compounds found in the sample were compared with spectra of synthetic references, (a) DMIP; (b) TMIP; and (c) IFP.

PhIP was much higher in the dry-heated chicken breast samples than the pork chop samples. MeIQ was detected in one sample, the heated meat juice from chicken breast, and this is the first time MeIQ has been detected in our model systems. MeIQ has earlier been identified in boiled pork juice (Lee, Lin & Chan, 1994) and in chemical model systems based on the precursor composition of chicken thigh, pork and fish (Pais et al., 1999). In addition, small amounts of Glu-P-2 was detected in the wet heating model system of pork chop meat juice (9 ng/g). Glu-P-2 has earlier been detected in smoke condensates of glutamic acid (Yamamoto et al., 1978), but we did not find any correlation with glutamic acid content in our study.

Interestingly, the pressed meat also produced HAs, though the meat had been depleted of meat juice before heating; the precursor content was still sufficient to produce HAs. In another study, 1–3 min microwave heating before frying beef patties was successfully used to release meat juice containing HA precursors from the meat, thus reducing the amount of HAs formed during cooking (Felton, Fultz, Dolbeare & Knize, 1994).

Large variations between triplicate determinations of the samples in Table 2 were observed, probably due to the severe heating conditions which produced a complex sample matrix. Thus, the second set of experiments was performed at a lower temperature, 175°C, and with meat juice only. The results are summarised in Table 3. PhIP was detected in all samples, but at a markedly higher level in the dry heated samples. This is in agreement with earlier results from our lab (Skog et al., 2000). The amount of PhIP in dry heated meat juice from chicken breast was about 10-fold higher than in other samples. When the meat surface is heated to a high temperature, it may partly be dried out, which will favour PhIP formation, and in meat cooking experiments, PhIP has been detected at very high levels, up to 480 ng/g, in pan-fried, oven-broiled or grilled/barbecued chicken breast (Sinha et al., 1995). Phenylalanine and leucine have earlier been shown to produce PhIP when dry-heated in model systems with creatine or creatinine, with and without the addition of sugar (Felton & Knize, 1990; Övervik, Kleman, Berg & Gustavsson, 1989). The total concentration of these amino acids were about 6 times lower in meat juice from roast beef and pork chop than chicken breast samples. In addition, the roast beef and pork chop samples contained about 4 to 16 times more glucose, which may explain the differences in PhIP

Table 1
Creatine, creatinine, glucose, free amino acids in meat juice from roast beef, pork chop and chicken breast (μmol/gdm)

Compound	Roast beef	Pork chop	Chicken breast
Creatine	340	277	152
Creatinine	2.4	6.4	0.5
Glucose	65	16	3.9
Free amino acids (total)	110	105	222
Aspartic acid	1.7	0.3	9.4
Threonine	2.3	3.3	11.5
Serine	3.5	5.7	16.1
Glutamic acid	2.6	3.0	26.3
Proline	1.9	10.3	8.0
Glycine	9.0	21.1	12.8
Alanine	28	4.1	23.7
Valine	3.2	0	9.1
Cysteine	0	1.3	0
Methionine	0.7	2.9	3.7
Isoleucine	1.7	12.2	7.7
Leucine	3.8	2.3	27.9
Tyrosine	1.5	2.6	2.7
Phenylalanine	1.2	3.6	4.5
Lysine	2.8	1.6	11.0
Histidine	1.3	2.8	7.2
Arginine	2.8	0	9.6
Aspargine	0.1	1.4	0
Glutamine	36	9.9	16.9
Tryptophan	4.8	0 .5	11.0
Taurine	14	15.8	2.8

Table 2
Heterocyclic amines (HAs) (ng/gdm) formed in the model system based on pressed meat and meat juice juice from pork chop and chicken breast heated at 200°C for 30 min (mean values ± S.D. are given)

HAs	Pork chop				Chicken breast			
	Dry		Wet		Dry		Wet	
	Pressed meat ($n=5$)	Meat juice ($n=3$)	Pressed meat ($n=3$)	Meat juice ($n=3$)	Pressed meat ($n=5$)	Meat juice ($n=3$)	Pressed meat ($n=3$)	Meat juice ($n=3$)
PhIP	24±31	36±62	nd[a]	1±1	300±270	280±420	nd	7±6
DMIP	160±200	200±110	75±18	nd	29±45	140±100	12±20	nd
TMIP	49±47	111±25	56±97	26±44	64±52	149±37	35±61	34±59
IFP	22±33	34±34	21±36	54±93	250±190	220±340	65±110	nd
MeIQx	21±6	100±64	460±460	360±620	15±34	nd	19±33	150±120
DiMeIQx	4±8	3±5	nd	nd	nd	nd	nd	nd
Norharman	130±280	36	870±690	450±780	370±240	63±93	550±950	520±870
Harman	2±4	nd	1280±2100	910±1600	121±117	16±28	1700±2900	1600±2700

[a] nd, not detected.

formation, as glucose in excess has been found to decrease the formation of PhIP in model systems (Skog & Jägerstad, 1991).

In the dry system, heated at 175°C/30 min, DMIP and TMIP were identified in all meat juices using LC/MS. DMIP was detected also in wet-heated samples. The precursors for DMIP and TMIP are unknown, but TMIP has been detected after dry-heating of threonine and creatine (Övervik et al., 1989). Threonine has also been suggested as a tentative precursor for DMIP, since TMIP is a methyl analogue of DMIP (Pais et al., 1999), but we did not find any correlation between threonine content and DMIP and TMIP formation.

IFP was identified in all dry-heated samples. In another study in which amino acids, creatine and glucose, at concentrations corresponding to those in beef, pork and chicken breast, were dry-heated at 225°C for 30 min, IFP was detected at levels comparable to PhIP (Pais et al., 1999). IFP can be produced from creatin/in/e and glucose (Pais et al., 2000), but glutamine and glutamic acid are the amino acids that increase the formation of IFP when heated together with sugars in a model system. In our samples, glutamine together with glutamic acid were among the most abundant free amino acids in the meat juices.

The data in Table 3 clearly show that the formation of TMIP and IFP was inhibited in the presence of water. We have earlier shown that less PhIP is formed in model systems based on bovine meat juice in the presence of water (Skog et al., 2000), and it now appears that water also has an inhibiting effect on the formation of the imidazopyridine TMIP and the imidazofuropyridine IFP. Results from another study also suggest that the formation of IFP is favoured by the same conditions that favour PhIP formation (Pais et al., 2000).

As expected, MeIQx formation was favoured by wet-heating, which is in accordance with previous results (Skog et al., 2000). Interestingly, the amounts of MeIQx in the pork chop samples were more than twice those in the roast beef samples and about 10 times those in chicken breast samples. In addition, 4,8-DiMeIQx was easily detected in the pork chop meat juice, also when dry-heated, while only very low levels were found in the other two meat juices. In addition, 7,8-DiMeIQx was detected at low levels in some samples. MeIQx is formed from several amino acids when heated with creatin/in/e and glucose in aqueous systems (Johansson, Fay, Gross, Olsson & Jägerstad, 1995; Skog et al., 1998). Creatine is a key precursor of IQx-derivatives (Jägerstad et al., 1983, 1984) and the lower creatine content in the chicken breast sample or a favourable ratio between amino acids, creatine and glucose, may explain this result. Using HPLC with UV detection, IQx was detected only in the wet-heated meat juice from roast beef but, using LC/MS, the presence of IQx was established also in the dry- and wet-heated meat juice from pork chop. IQx, MeIQx and DiMeIQx have been detected in both chemical model systems (Arvidsson et al., 1997; Pais et al., 1999) and model systems based on meat juice from beef (Arvidsson et al., 1999; Skog et al., 2000). IQ and MeIQ were not detected in any of the samples. IQ and MeIQ have been detected earlier in boiled pork juice (Lee et al., 1994). IQ has been detected in dry-heated model reactions with precursors in amounts corresponding to different meats (Pais et al., 1999) and in serine or proline model systems when heated with creatinine (Felton & Knize, 1990; Knize et al., 1998).

Norharman and Harman were detected at higher levels in the wet-heated samples of roast beef and pork chops than in the dry-heated samples which is in agreement with earlier results (Skog et al., 2000). The opposite result was found for chicken breast. Both in the wet and the dry system, more Harman than Norharman was formed. Tryptophan is a precursor of Norharman and Harman (Sugimura, Nagao & Wakabayashi, 1982), but in our study no correlation was found between the tryptophan content in the unheated samples and Norharman/Harman formation.

Table 3
Heterocyclic amines (ng/dm) formed in the model system based on pressed meat juice from roast beef, pork chop and chicken breast heated at 175°C for 30 min (mean values ± S.D. are given; $n = 3$)

	Roast beef (dry)	Roast beef (wet)	Pork chop (dry)	Pork chop (wet)	Chicken breast (dry)	Chicken breast (wet)
PhIP	119±2	75±7	159±17	29±4	1490±4	21±1
DMIP[a]	443±187	380±98	357±132	829±162	1070±225	379±34
TMIP[a]	624±232	nd[b]	384±203	nd	320±69	nd
IFP[a]	81±7	nd	158±61	nd	207±76	nd
IQx[a]	nd	58±40	62±12	130±44	nd	nd
MeIQx	25±6	186±17	243±67	557±86	17±2	40±1
4,8-DiMeIQx	nd	3±0.5	156±46	77±4	nd	d[c]
7,8-DiMeIQx	nd	3±1	nd	nd	15±1	nd
Norharman	34±17	429±78	156±67	213±47	258±185	121±13
Harman	187±52	1690±230	588±264	806±167	722±275	451±22

[a] Identified and quantified using LC/MS.
[b] nd, not detected.
[c] d, detected, but not quantified, due to co-eluting impurities.

Trp-P-1 and Trp-P-2 were not detected in our experiments, which is in agreement with earlier results from a model system based on meat juice from beef, where Trp-P-1 and Trp-P-2 were only detected after the addition of tryptophan (Skog et al., 2000).

3.2. Influence of extraction solvent on recovery rates

During the study, the efficiency of EtAc and DCM for extraction of spiked samples was evaluated, and EtAc was found to be the better extraction solvent. For both extraction solvents, the recovery rates for IQ were only about 25–30% and, for MeIQ and IQx, DiMeIQx and MeIQx, around 70–100%, and these did not vary much with the different solvents. Norharman and Harman were markedly better extracted with EtAc; the recovery rate for the extraction with DCM was only 1–2% and, with EtAc, 100%. For Trp-P-1 and Trp-P-2, recovery rates were around 60% (Trp-P-1) and 50% (Trp-P-2) with DCM compared with 25% (Trp-P-1) and 50% (Trp-P-1) for EtAc. The recovery rate of DMIP increased from 12% with DCM to 32% with EtAc. Thus DMIP was better extracted with EtAc, which is in accordance with the results from another study on formation of HAs in a model system (Pais et al., 1999). A changeover to other solvents has been made in other fields of extraction; for example, nitrous cyclic herbicide and pesticide residues have successfully been extracted and analysed at ppm levels using EtAc instead of DCM (Juhler, 1997; Nordmeyer & Thier, 1999). Furthermore, unspiked samples were extracted with EtAc, DCM or their combination, and the extraction of, for example, MeIQx was markedly improved with a mixture of DCM and EtAC. Probably, some of the MeIQx was bound very strongly to the sample matrix, and these bonds were more efficiently broken with a mixture of DCM and EtAc. This considerable difference in the extractability of MeIQx from unspiked and spiked samples suggests that some substances formed during cooking bind to the HAs. The binding effect may be due to melanoidins (melanin-resembling polymers) formed via the Maillard reaction during the cooking of meat (Baltes, 1990; Obretenov, 1993), since other studies have shown that HAs bind to melanin (Brittebo, Skog & Jägerstad, 1992). Our experiments show that a substantial proportion of the HAs remains in the sample matrix after extraction with DCM or EtAc, and that a mixture of DCM and EtAc may increase the extraction yield for some HAs.

4. Conclusions

Three HAs that have recently been reported in the literature, DMIP, TMIP and IFP, were detected in several model systems based on meat juice from roast beef, pork chop and chicken breast. The highest levels of PhIP and IFP were detected in dry heated meat juice from chicken breast. IQx, DiMeIQx and MeIQx were found at highest levels in heated meat juice from pork chop. Thus, precursor composition has a pronounced effect on the formation of HAs. The formation of TMIP, IFP and PhIP was favoured by dry conditions, while the formation of MeIQx was favoured by wet conditions, which means that the presence of water clearly affects HA formation.

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