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Effects of heating conditions and additives on the formation of heterocyclic amines with reference to amino-carbolines in a meat juice model system

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Abstract

Common cooked meat and fish products contain heterocyclic amines. Lyophilised bovine meat juice of known composition has been studied in a model system to increase knowledge of the conditions leading to the formation of, especially, the amino-carboline-type heterocyclic amines. The reaction conditions included dry and aqueous heating (at 175°C for 10 min or at 200°C for 30 min) in open and closed vials, with the addition of iron(II)sulphate, creatinine, glucose or tryptophan. After dry-heating the meat juice at 200°C for 30 min, A$\propto$C was clearly identified, together with traces of Trp-P-1 and Trp-P-2. The non-mutagenic β-carbolines harman and norharman formed easily, and the yield increased with increasing temperature, absence of water and the addition of tryptophan. Other heterocyclic amines, e.g. IQx, MeIQx, and PhIP were also identified. Aqueous heating favoured IQx and MeIQx formation, while dry heating favoured PhIP formation.

Keywords: Heterocyclic amines; Amino-carbolines; Imidazo-azaarenes; Food mutagens; Model system; Meat juice; Tryptophan; Iron; Creatinine

1. Introduction

Heterocyclic amines present in fried, broiled or barbecued foods can be divided into two main classes: (i) amino-carbolines and (ii) amino-imidazo-azaarenes (AIAs) (Hatch, Felton, Stuermer & Bjeldanes, 1984; Sugimura, Wakabayashi, Nagao & Esumi, 1993). Varying numbers and positions of methyl groups add to the number of derivatives within each class (Fig. 1). The amino-carbolines are further sub-grouped into α-, β-, γ- and δ-carbolines represented by, e.g. α-carbolines: 2-amino-9H-pyrido[2,3-*b*]indole (AαC) and 1-methyl-2-amino-9H-pyrido[2,3-*b*]indole (MeAαC), β-carbolines: 1-methyl-9H-pyrido[3,4-*b*]indole (harman) and 9H-pyrido[3,4-*b*]indole (norharman), γ-carbolines: 3-amino-1,4-dimethyl-5H-pyrido[4,3-*b*]indole (Trp-P-1) and 3-amino-1-methyl-5H-pyrido[4,3-*b*]indole (Trp-P-2), and, δ-carbolines: 2-amino-6-methyl-dipyrido[1,2-a:3′,2′-*d*]indole (GluP-1) and 2-amino-dipyrido[1,2-*a*:3′,2′-*d*]indole (Glu-P-2) (Hatch et al., 1984; Sugimura, Nagao & Wakabayashi, 1982).

The amino-carbolines, except for the β-carbolines (harman and norharman) are sometimes referred to as pyrolytic mutagens (Hatch et al., 1984; Sugimura et al., 1982), as they were originally isolated from smoke condensates collected from cigarettes and from pyrolysed single amino acids, e.g. tryptophan, glutamic acid, lysine, phenylalanine and ornithine, or from pyrolysed proteins such as casein, albumin, gluten or soybean globulin. Pyrolysis occurs at temperatures well above 300°C, and produces many reactive fragments through radical reactions. These fragments are believed to condense to form new heterocyclic structures, and pyrolytic mutagens might be formed via free-radical reactions, but little work has in fact been done to elucidate the mechanisms and pathways leading to their formation.

The AIA class of heterocyclic amines, on the other hand, has been rather extensively studied concerning precursors, reaction pathways and conditions (for ref.,

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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
Norharman	9H-pyrido[4,3-*b*]indole, CAS no: 244-63-3
Harman	1-methyl-9H-pyrido[4,3-*b*]indole, CAS no: 486-84-0
Trp-P-1	3-amino-1,4-dimethyl-5H-pyrido[4,3-*b*]indole, CAS no: 62450-06-0
Trp-P-2	3-amino-1-methyl-5H-pyrido[4,3-*b*]indole, CAS no: 62450-07-1
AαC	2-amino-9H-pyrido[2,3-*b*]indole, CAS no: 26148-68-5
MeAαC	2-amino-3-methyl-9H-pyrido[2,3-*b*]indole, CAS no: 68006-83-7
AIA	amino-imidazo-azaarene
CBA	Carboxypropyl silica
PRS	Propylsulphonic acid silica

see Felton, Knize, Skog, Jägerstad & Wakabayashi, in press; Jägerstad, Skog, Arvidsson & Solyakov, 1998; Skog, Johansson & Jägerstad, 1998). Several of the heterocyclic amines have been proven carcinogenic in long-term animal experiments, and an increasing number of studies indicate that humans absorb and metabolise heterocyclic amines, resulting in DNA adduct formation (see recent reviews by Felton, Malfatti, Knize, Salmon, Hopmans & Wu, 1997; Sugimura, 1997). As heterocyclic amines are candidates in the aetiology of human cancer (International Agency for Research on Cancer, IARC, 1993), the search for ways to minimise their intake by limiting their occurrence in cooked foods is very important.

Many amino-carbolines have been detected in foods prepared at 100–225°C, e.g. in meat extracts and bouillons (Galceran, Moyano, Puignou & Pais, 1996; Galceran, Moyano, Pais & Puignou, 1996; Holder, Cooper, Churchwell, Doerge & Thompson, 1996; Pais, Moyano, Puignou & Galceran, 1997a,b; Skog, Solyakov, Arvidsson & Jägerstad, 1998; Solyakov, Skog & Jägerstad, 1999), and in meat and fish dishes (see review by Brockstedt & Pfau, 1998; Gross, Turesky, Fay, Stillwell, Skipper & Tannenbaum, 1993; Holder, Cooper, Churchwell, Doerge & Thompson, 1996; Knize, Sinha, Salmon, Mehta, Dewhirst & Felton, 1996; Skog, Steineck, Augustsson & Jägerstad, 1995; Skog, Augustsson, Steineck, Stenberg & Jägerstad, 1997; Skog, Solyakov, Arvidsson & Jägerstad, 1998; Thiébaud, Knize, Kuzmicky, Felton & Hsieh, 1994; Thiébaud, Knize, Kuzmicky, Hsieh & Felton, 1995; Wu, Wong, Lee, Lee, Shi & Ong, 1996). There is thus a need for knowledge concerning the pathways and mechanisms leading to the formation of amino-carbolines in the temperature range

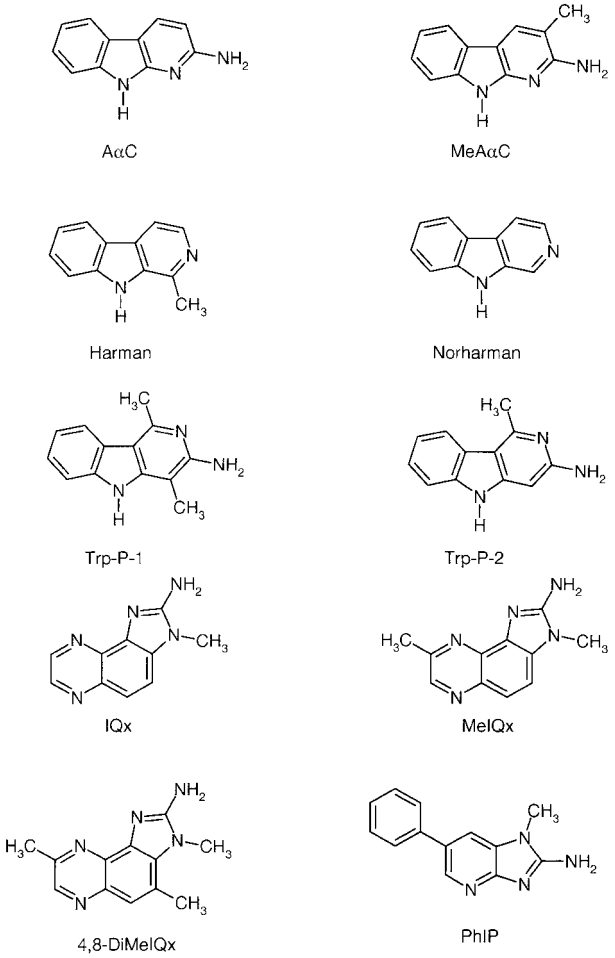

Fig. 1. Structures of some of the heterocyclic amines studied. Amino-α-carbolines: AαC and MeAαC, amino-β-carbolines: harman and norharman, amino-γ-carbolines: Trp-P-1 Trp-P-2. AIAs: IQx, MeIQx, 4,8-DiMeIQx and PhIP.

typically reflecting boiling, frying, deep-frying and roasting. Some aminocarbolines have been identified in model systems. Trp-P-1 was identified in a beef-derived supernatant model system, after boiling for 30 min (Taylor, Fultz & Knize, 1986), and Trp-P-1 and Trp-P-2 were detected after heating creatinine, glucose and tryptophan or isoleucine in water at 180°C for 10 min (Johansson, Fay, Gross, Olsson & Jägerstad, 1995). Several amino acids or mixtures of amino acids produced norharman and harman when heated in water at 100–225°C together with creatine and glucose (Arvidsson, van Boekel, Skog & Jägerstad, 1997; Johansson et al., 1995). A model system based on meat juice was recently developed and applied to kinetic studies of the formation of IQx-derivatives and 2-amino-1-methyl-6-phenylimidazo-[4,5-*b*]pyridine (PhIP) in the temperature range 100–225°C (Arvidsson, van Boekel, Skog, Solyakov & Jägerstad, 1999). The only amino-carbolines identified were harman and norharman.

In the present study, we focused on conditions favouring the formation of amino-carbolines. A meat juice model system was used, and the reaction conditions comprised dry and aqueous heating in open and closed vials, and the addition of iron(II)sulphate, creatinine, glucose, or tryptophan. Heterocyclic amines of both classes (amino-carbolines and AIAs) were identified and quantified.

2. Materials and methods

2.1. Chemicals

All chemicals and solvents were of HPLC or analytical grade. Water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA). Heterocyclic amines used as analytical standards, Trp-P-1, Trp-P-2, AαC, MeAαC, harman, IQ (2-amino-3-methylimidazo [4,5-*f*]quinoline), IQx (2-amino-3-methylimidazo[4,5-*f*] quinoxaline), MeIQ (2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline), 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), and PhIP, were purchased from Toronto Research Chemicals (Toronto, Canada), and norharman was purchased from Aldrich (Steinhem, Germany). According to the producers, the chemical purity of the reference compounds was higher than 99%. This was confirmed using HPLC with UV detection and multi-component analysis for each of the reference compounds, as previously described (Skog et al., 1997). A mixture of the different heterocyclic amines (2 ng of each compound/μl) in methanol:0.01 M triethylamine pH 3.2 (1:1) was used as spiking solution. A solution of 2-aminofluorene (100 ng/μl) in methanol:0.01 M triethylamine pH 3.2 (1:1) was used as an internal standard. D-glucose, creatinine and L-tryptophan were obtained from Sigma (LabKemi AB, Lund, Sweden) and iron(II)sulphate (henceforth referred to as iron) from Merck AG (Darmstadt, Germany). Materials for solid-phase extraction (Extrelut, PRS, C_{18}, and CBA) were obtained from Merck and Analytichem International (Sorbent, Västra Frölunda, Sweden).

2.2. Meat juice

Meat juice was prepared from bovine roast beef (*Musculus longissimus dorsi*), as described previously (Arvidsson et al., 1999), freeze-dried and stored at −18°C. The freeze-dried meat juice was analysed for pH, dry matter content, free amino acids, creatine/creatinine and glucose, as previously described (Arvidsson et al., 1997). All analyses were performed in duplicate.

2.3. Model system

In the first set of experiments, to examine the effect of water on the formation of heterocyclic amines, concentrated meat juice was prepared prior to use by vigorously mixing freeze-dried meat juice with distilled water in the proportions 1:2 (freeze-dried meat juice: water) by weight. About 1.7 g of the mixture were transferred into quartz test-tubes and heated in a thermostat-controlled oil-bath at 175°C for 10 min (Arvidsson et al., 1999). Some test-tubes were sealed before heating (closed aqueous system) while others were not (open aqueous system). Freeze-dried meat juice was also heated in open test tubes at 175°C for 10 min or at 200°C for 30 min without being mixed with water (open dry system). After heating, the test-tubes were immediately cooled in an ice-bath. The design of the experiment is illustrated in Table 1. Single analysis was applied to the results given in Table 1 in light of the low variation seen in earlier control samples at our laboratory (Arvidsson et al., 1999).

In the second set of experiments, meat juice was mixed with tryptophan, to final concentrations of 2, 2.5 or 5 times the original concentration, or glucose, (2.5 or 5 times the original concentration), or one of the following: iron, creatinine, tryptophan + iron, or tryptophan + creatinine (5 times the original concentration). The experimental protocols are given in Tables 2 and 3. The test-tubes were heated at 175°C for 10 min (closed aqueous system) or at 200°C for 30 min (open dry system). The experiments were performed in triplicate.

2.4. Identification and quantification of heterocyclic amines

Heated samples were extracted and purified according to the solid-phase extraction method described by Gross and Grüter (1992) with some minor modifications (Knize et al., 1995; Fay, Ali & Gross, 1997). Extraction yields were determined by adding 50 μl of the spiking mixture to

one of three samples from each heating session before extraction. Most samples were subjected to additional purification using CBA columns (Solyakov et al., 1999).

Purified samples were analysed using reversed-phase HPLC. The effluent was monitored with a photodiode array UV detector in the wavelength range 224–367 nm (Varian 9065, Polychrome) and a programmable fluorescence detector (Varian LC 9070) (Skog et al., 1997). The identities of the peaks were established by comparing retention times of peaks in UV chromatograms with synthetic references, and by comparing their UV spectra. The heterocyclic amines were quantified by comparing peak areas of samples with areas of peaks from known amounts in reference solutions from UV chromatograms at 263 nm (IQ, IQx, MeIQ, MeIQx, 7,8-DiMeIQx, 4,8-DiMeIQx, Trp-P-1 and Trp-P-2), at 302 nm (norharman and harman), at 316 nm (PhIP) or at 344 nm (AαC and MeAαC). The amounts of heterocyclic amines were corrected for incomplete recovery and expressed as ng/g freezedried meat juice.

3. Results

3.1. Water and the formation of heterocyclic amines

The results regarding the effect of water are summarised in Table 1. Heating the meat juice mixed with water in the *closed aqueous system* at 175°C for 10 min resulted in the formation of IQx, MeIQx, PhIP, harman and norharman. The IQx-derivatives 4,8-DiMeIQx and 7,8-DiMeIQx were also identified but, due to co-eluting

Table 1
Effect of heating conditions on the formation of heterocyclic amines (ng/g dry meat juice) in model systems

Heating conditions model system		Temperature (°C)	Time (min)	IQx	MeIQx	PhIP	Harman	Norharman	AαC
Closed	Aqueous	175	10	65	110	14	300	210	ND[a]
Open	Aqueous	175	10	ND	39	ND	100	38	ND
Open	Dry	175	10	ND	trace	200	810	290	ND
Open	Dry	200	30	ND	36	350	990	410	17

[a] ND = not detected.

Table 2
Formation of heterocyclic amines in a closed aqueous system heated at 175°C for 10 min (ng/g dry meat juice)

Additives	IQx	MeIQx	PhIP	Harman	Norharman	AαC
None	65	110	14	300	210	ND[c]
Creatinine×5[a]	170	270	ND	270	110	ND
Iron×5	ND	160	ND	270	110	ND
Trp[b]×2	120	150	trace	7000	1400	ND
Trp×5	93	160	ND	21000	540	ND
Trp×5 + iron×5	140	200	ND	21000	4200	ND
Trp×5 + creatinine×5	160	330	ND	21000	3200	ND

[a] ×5 means that the compound was added to a final concentration 5 times the original.
[b] Tryptophan.
[c] ND = not detected.

Table 3
Formation of heterocyclic amines in an open dry system heated at 200°C for 30 min (ng/g dry matter)

Additives	IQx	MeIQx	4,8-DiMeIQx	7,8-DiMeIQx	PhIP	Harman	Norharman	AαC
None	ND	36	D[c]	ND[d]	350	990	410	17
Glucose×2.5[a]	ND	D	trace	ND	340	1100	480	ND
Glucose×5	ND	D	trace	ND	420	950	450	ND
Trp[b]×2.5	ND	D	D	ND	670	2900	3000	ND
Trp×5	ND	D	D	ND	690	5500	6100	ND

[a] ×2.5 means that the compound was added to a final concentration 2.5 times the original.
[b] Tryptophan.
[c] D = detected but not quantified due to co-eluting interferences.
[d] ND = not detected.

substances, they could not be properly quantified in all cases. In the *open aqueous system*, the amount of heterocyclic amines was markedly decreased. IQx and PhIP were not detected at all. After heating the meat juice in the *open dry system* at 175°C for 10 min, the only IQx-derivative identified was MeIQx. Interestingly, the amount of PhIP increased about 15-fold, and the amount of harman doubled, and there was an increase in norharman. Following open dry heating at 200°C for 30 min, MeIQx was detected at similar concentrations to those in the open aqueous system at 175°C/10 min, and the amounts of PhIP, harman, and norharman were further increased. For the first time, AαC was identified in our model system, as shown in Fig. 2. In this set of experiments, IQ, MeIQ, and MeAαC were not detected in any of the samples, and Trp-P-1, Trp-P-2 were only tentatively identified using a fluorescence detector. Norharman (approx. 3 ng/g dry matter) and harman (approx. 5 ng/g dry matter) were identified as the only heterocyclic amines in the unheated meat juice.

3.2. Addition of possible precursors for the formation of heterocyclic amines

When creatinine was added to the *closed aqueous model system*, the amounts of IQx and MeIQx increased, but the amount of norharman decreased, and PhIP was not detected (Table 2). With the addition of iron, the formation of MeIQx increased, while the amounts of harman and norharman decreased, and neither IQx nor PhIP was detected. The addition of tryptophan increased the formation of IQx and MeIQx, harman and norharman, but the amount of PhIP decreased. With the addition of tryptophan at 5 times the original level, the concentration of harman increased more than 70-fold, and this dramatic increase was also found upon the addition of tryptophan in combination with iron or creatinine. Iron or creatinine, together with tryptophan, enhanced the formation of norharman and MeIQx more than when the compounds were added separately.

In the *open dry system* (200°C/30 min), the addition of glucose or tryptophan reduced the formation of IQx and MeIQx (Table 3). Glucose had no or only a slightly enhancing effect on the formation of PhIP, harman and norharman. The addition of tryptophan, however, almost doubled the amounts of PhIP, and increased the amounts of harman and norharman by 3–15 times. No AαC was detected when glucose or tryptophan was added to the system. Trp-P-1 and Trp-P-2 were detected using fluorescence, and IQ, MeIQ, AαC or MeAαC were not detected when any of the chemicals were added to the model system.

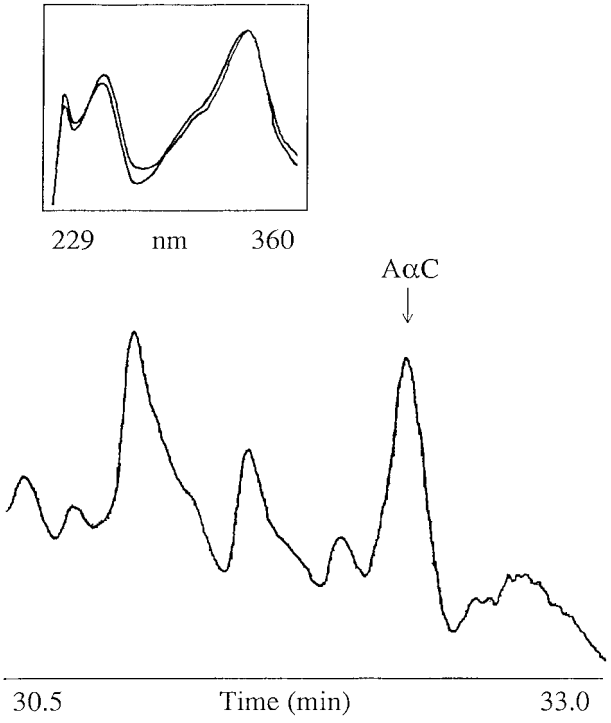

Fig. 2. Expanded region of a chromatogram from the HPLC analysis of meat juice dry-heated at 200°C for 30 min. The plot shows the UV absorption at 344 nm. The peak corresponding to AαC is indicated. The insert shows the on-line recorded UV spectrum of AαC in the same sample compared with the spiked sample spectrum.

3.3. Chemical analysis and pH

The yield of fresh meat juice obtained from the meat was 12.5% by weight corresponding to the amount of liquid not bound inside the protein network of meat. The fresh and the freeze-dried meat juice had dry matter contents of 12.2% and 95.0%, respectively. The contents of free amino acids, creatine/creatinine and glucose are given in Table 4. The pH of the freshly prepared meat juice was 5.6.

3.4. Quality assurance of heterocyclic amine analysis

Average recoveries from the purification stage were >70% for the IQ and IQx compounds, >60% for PhIP, >50% for harman, norharman, Trp-P-1 and Trp-P-2, and >40% for AαC and MeAαC. In general, detection limits were about 1.5 ng per injection; however, depending on the complex matrices, the limit was sometimes higher. The absorbance from the UV diode array detector showed linearity in the range used, i.e. up to 1500 ng per injection. In general, triplicate values varied by about ±5%.

4. Discussion

Dry heating of the lyophilised meat juice at 200°C for 30 min was the only condition for which amino-α-carboline,

Table 4
Free amino acids, creatin/in/e, and glucose in freeze-dried bovine meat juice

Compound	(μmol/g dry matter)
Free amino acids (total)	110
Aspartic acid	1.7
Threonine	2.3
Serine	3.5
Glutamic acid	2.6
Glutamine	36
Proline	1.9
Glycine	9.0
Alanine	28
Valine	3.2
Methionine	0.72
Isoleucine	1.7
Leucine	3.8
Tyrosine	1.5
Phenylalanine	1.2
Lysine	2.8
Histidine	1.3
Tryptophan	4.8
Arginine	2.8
Taurine	14
Carnosine	400
Glucose	65
Creatine	340
Creatinine	2.4

i.e. AαC, was clearly identified. To the best of our knowledge, this is the first report on the formation of AαC in a model system heated at a normal cooking temperature. Furthermore, the γ-amino-carbolines, Trp-P-1 and Trp-P-2, were tentatively detected using fluorescence, and these compounds were detected in the aqueous model system heated at 175°C for 10 min with the addition of tryptophan. In contrast, a pilot study in our laboratory showed that when minced meat was fried in a pan for 6 min at 200°C, AαC and traces of Trp-P-1 and Trp-P-2 were formed, but when meat juice from the same muscle was heated in a closed aqueous system at 175°C for 10–20 min, none of these compounds could be detected. These contradictory results are difficult to explain, although the heating conditions were not exactly the same. Literature data report the presence of amino-carbolines in common cooked foods (Table 5), although less frequently than the AIA class of heterocyclic amines (See the reviews by de Meester, 1995; Layton et al., 1995; Robbana-Barnat, Rabache, Rialland & Fradlin, 1996; Skog, Johansson et al., 1998; Sugimura, 1997). This means that precursors for amino-carbolines are present in muscle, and probably also in meat juice. The absence of amino-α- and γ-carbolines in meat juice heated with water at 175°C in a closed model system implies that water or some volatile compound acts as an inhibitor. On the other hand, Trp-P-2, but not Trp-P-1 was identified in an aqueous supernatant fraction of beef heated at around 100°C for 30 min (Taylor et al., 1986). Moreover, in a recent study (Solyakov et al., 1999), Trp-P-1, AαC and MeAαC were identified in a process flavour of pure vegetable origin, heated below 105°C for 1–5 h, which indicates that precursors for the formation of amino-α- and γ-carbolines are not exclusively found in muscle, and that there are several pathways for their formation.

The non-mutagenic amino-β-carbolines, harman and norharman, formed easily after both dry and aqueous heating of the meat juice. Among the additives investigated, tryptophan enhanced the yield of harman and norharman dramatically, which indicates that tryptophan is their precursor. After aqueous heating, a 5-fold increase in tryptophan levels resulted in a 70-fold increase in the yield of harman. Norharman increased in yield by about 15-fold when tryptophan and iron were added in combination (5 times original concentrations). Harman and norharman were detected in fried meat, but were not detected when creatine, glucose and a mixture of 16 amino acids were dry heated at 225°C for 30 min; however, tryptophan was not included (Pais, Salmon, Kinze & Felton, 1999). That tryptophan is a potent precursor of harman and norharman has previously been reported by Sugimura et al. (1982). In a study by Yaylayan, Jocelyn Paré, Laing, Sporns (1990), electron impact mass spectral fragmentation was used in an attempt to mimic high-temperature decomposition in solutions. It was demonstrated that Amadori rearrangement products of tryptophan were fragmented and rearranged into two types of β-carbolines, one of which was norharman. Harnan and norharman seem thus to form easily in meat-derived products, and interestingly, they were detected at very low levels even in the unheated meat juice, indicating possible formation already in vivo. They have previously been demonstrated in an aqueous meat juice model system heated at boiling temperature, 100°C, and are always found at the highest concentrations among all heterocyclic amines in the temperature range 100–225°C (Arvidsson et al., 1999). In a recent screening study on process flavours and process flavour ingredients, bouillon concentrates and a pan residue sample (Solyakov et al., 1999), harman/norharnan were found to be present in 15/16 out of a total of 17 samples in the range of 5–900 ng/g, thus exceeding the concentration of mutagenic heterocyclic amines by factors 10–100. Harman and norharman by themselves are not mutagenic in the Ames/Salmonella test, due to the lack of an exocyclic amino group, but they increase the mutagenic activity of Trp-P-1 and Trp-P-2 (Sugimura et al., 1982). Moreover, norharman in combination with non-mutagenic aromatic amines, such as aniline and *o*-toluidine, produce mutagenic activity and DNA adduct formation (Wakabayashi, Totsuka, Fukutome, Oguri, Ushiyama & Sugimura, 1997). Harman and norharman have also been reported to act as neurotoxins and enzyme inhibitors, for instance towards

Table 5
Quantified amounts of amino-α,β- and γ-carbolines (ng/g) in foods cooked at or below 200°C

Sample	Cooking method	Temperature (°C)	Time (min)	AαC	MeAαC	Harman	Norharman	Trp-P-1	Trp-P-2	Reference
Beef steaks	Fried	190	6–13	3.2–8.9		3–4.8	8.7–19.3			Gross, 1990
Salmon	Pan-broiled	200	6–24	ND-9		2–34	8–28			Gross and Grüter, 1992
Bacon strips	Grilled	170	12–16	ND[a]		ND-22	ND-30			Gross et al., 1993
Bacon strips	Microwaved		3	0.1		ND	3.3			Gross et al., 1993
Beef	Fried	198	12	0–21						Thiébaud et al., 1995
Chicken, pan residue	Roasted	150	30			D[b]	D	0.02	ND	Skog et al., 1995
Meat sauce	Fried	200	6			D	D	0.7	0.6	Skog et al., 1995
Meat sauce	Fried	175	6			D	D	0.3		Skog et al., 1995
Chicken	Fried	190	2×6	ND	6.8	ND	ND	3.7	2.9	Brockstedt and Pfau, 1998
Turkey	Fried	190	2×6	18.7	ND	12.0	16.5	ND	5.1	Brockstedt and Pfau, 1998
Pork chop, pan residue	Roasted	175	60	0.04	0.08	0.04	0.04	ND	ND	Skog, Solyakov et al., 1998
Meat loaf, pan residue	Roasted	175	45	0.28	ND	0.04	0.2	0.08	ND	Skog, Solyakov et al., 1998
Meat loaf, pan residue	Roasted	175	45	ND	ND	14	52.6	1.7	ND	Solyakov et al., 1999

[a] ND = not detected.
[b] D = Detected but not quantified.

monoamine oxidases and mono-oxygenases (de Meester, 1995; Kuhn, Müller, Grosse & Rommelspacher, 1996). Therefore, the presence of harman and norharman in cooked foods should not be ignored, and model systems are available to study the conditions for their formation, as evidenced by the present study and others (Arvidsson et al., 1997, 1999; Solyakov et al., 1999).

Although the amino-carbolines had the highest priority regarding quantification in this study, the AIA group of heterocyclic amines was analysed simultaneously. IQx, MeIQx and PhIP were the major AIA derivatives found. The addition of creatinine, tryptophan, or iron enhanced the formation of MeIQx in the aqueous system, but inhibited the formation of PhIP. The addition of iron has earlier been shown to increase the yield of MeIQx in an aqueous model system (Johansson & Jägerstad, 1996). In contrast to our results, the amounts of both MeIQx and PhIP were found to increase when myoglobin, containing iron (Fe^{2+}), was applied to the surface of meat before frying (Murkovic, Steinberger & Pfannhauser, 1998).

Maillard reactions are involved in the formation of IQ- and IQx-type heterocyclic amines (see the review by Skog, Johansson et al., 1998). However, the addition of glucose did not increase the yield of these heterocyclic amines during dry heating, in contrast to previous studies carried out in aqueous model systems where glucose at low doses increased, but at high doses inhibited the formation of MeIQx and PhIP (see Skog, Johansson et al., 1998). Aqueous heating favoured IQx and MeIQx formation compared with dry heating. In contrast, Eichner and Shuirman (1994), reported a 2- or 3-fold decrease in the yield of MeIQx when meat extracts containing increasing amounts of water (between 7.4 and 52%) were heated at 100 and 120°C for 4 and 2 h, respectively. The Maillard reaction exhibits rate maxima at intermediate to high water activity (Labuza & Saltmarch, 1981), and therefore, it seems that dry heating is not a good model for studying the influence of the Maillard reaction.

Interestingly, the yield of PhIP increased dramatically in the dry model system, in contrast to the IQx-compounds. PhIP formation has earlier been demonstrated after dry heating the pure chemicals phenylalanine and creatinine at 200°C for 1 h (Felton & Knize, 1990; Övervik, Kleman, Berg & Gustafsson, 1989), or a mixture of amino acids, including phenylalanine, creatine and glucose (Pais et al., 1999). A possible explanation for the differences in PhIP formation, related to water content, could be in terms of water and free radical quenching (Gopala Krishna & Prabhakar, 1992; Nawar, 1996), and our results indicate that free radicals might be of importance for the formation of PhIP.

This is the first study in which an aqueous and a dry model system have been compared using the same samples. Notably less AIAs were detected in the open system. This may be due to the evaporation of key intermediates essential for the formation of heterocyclic amines or to the evaporation of heterocyclic amines. Heterocyclic amines are not very volatile, but some of them have been detected in small amounts in cooking fumes (Thiébaud et al., 1995; Vainiotalo, Matveinen & Reunanen, 1993). The pressure inside the test-tubes may have influenced the formation, as suggested by Jackson and Hargraves (1995), although they did not propose a mechanism. However, earlier model experiments at our laboratory have not indicated significant variations in the yield of heterocyclic amines using open test-tubes with water:diethylene glycol or closed vials with water (Johansson, Skog & Jägerstad, 1993). Our results show that water has a considerable influence on the formation of heterocyclic amines. The results are in line with published

data on model systems based on chemicals heated with and without water indicating differences in yield and species of heterocyclic amines formed (see the review by Skog, Johansson et al., 1998).

In studies involving the frying of meat, many parameters, such as heat and mass transfer, vaporisation of water and crust formation, are difficult to control. In our model system, the heat transfer through the wall of the test-tubes was efficient, giving a temperature of 200°C within 30 s, and the temperature in the oil bath varied by only ±0.1°C (Arvidsson et al., 1997). Model systems are both versatile and easy to control and the results, regarding precursors and reaction conditions, have been shown to agree between chemical-based and meat-based systems (Arvidsson et al., 1997; Felton, Pais, Salmon & Knize, 1998; Pais et al., in press). Moreover, the results obtained with model systems agree with realistic cooking in terms of yield and pattern of heterocyclic amines (Knize, Shen & Felton, 1988). The data from the present study are very interesting considering that during frying, baking/roasting, or grilling/barbecuing, water is generally lost from the food item, resulting in decreasing water content from the inner to the outer parts, and a relatively dry surface, which generally contains about 50% water (Skog, Jägerstad & Lauser Reuterswärd, 1992; Skog, 1993). These physical and chemical changes affect the mass and heat transport, and the results from our model system can be used for future kinetic studies in realistic meat cooking experiments.

5. Conclusions

To conclude, the present study confirmed tryptophan to be an important precursor of the nonmutagenic amino-β-carbolines harman and norharman, which were very easily formed at normal cooking temperatures. These compounds were also demonstrated in unheated lyophilized meat juice. Tryptophan did not enhance the formation of amino-α- or γ-carbolines, and inhibited PhIP formation, but enhanced the formation of IQx compounds. Dry heating (200°C/30 min) produced AαC, and this is the first model system to have shown the formation of AαC at a normal cooking temperature. The presence of water had a pronounced effect on the species of heterocyclic amines: dry heating favoured the formation of PhIP and AαC, while aqueous heating favoured the formation of amino-β-carbolines and IQx compounds. Considering the fact that water is evaporating continuously during cooking (and thus the water content in the outer part of the meat is decreasing), greater attention should be paid to the role of water in the yield of various heterocyclic amines in cooked muscle foods. The model systems used here, with well-controlled and reproducible reaction conditions, allow studies on the effects of water, various precursors, and other enhancing or inhibiting compounds, on the formation of heterocyclic amines.

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