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Review

Screening for heterocyclic amines in chicken cooked in various ways

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Summary

Chicken cooked under well-controlled conditions and commercial chicken products were screened for heterocyclic amines (HAs). Chicken samples were boiled, deep-fried, pan-fried, oven-roasted, cooked in an unglazed clay pot or in a roasting bag in the oven, and oven broiled. 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (4,8-DiMeIQx), 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), 1-methyl-9*H*-pyrido[3,4-*b*]indole (harman) and 9*H*-pyrido[3,4-*b*]indole (norharman) were identified in several samples. Chicken cooked at low temperatures contained low amounts of HAs. In pan-fried chicken breasts, MeIQx was detected in amounts below 2 ng/g, 4,8-DiMeIQx below 0.6 ng/g, and PhIP in amounts up to 38 ng/g. Harman and norharman were detected in almost all samples (below 15 ng/g). In skin from a commercially barbecued chicken, MeIQx, 4,8-DiMeIQx and PhIP were detected, while only traces of MeIQx were detected in the meat. MeIQx was detected in a commercial chicken flavour, 0.1 ng/ml. No HAs were detected in pan-fried chicken liver. The results show that the content of HAs in chicken cooked in various ways is low if prepared at low temperatures, and increases with increasing cooking temperature. PhIP formation seems to start accelerating at cooking temperatures around or above 200 °C. Colour development increases with cooking temperature, but no correlation with HA content was observed. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Heterocyclic amines; Chicken; Mutagens; Cooked food; PhIP; MeIQx

1. Introduction

Food is essential to provide sustenance but may also be an important factor in the genesis of human diseases, for example, cancer (Doll and Peto, 1981). Several epidemiological studies have shown a correlation between the intake of fried, broiled or roasted (red) meat and the development of cancer, while other studies have found no reliable correlation (for review see Norat and Riboli, 2001). It has been suggested that heterocyclic amines (HAs) in cooked foods play a role in the aetiology of human cancer. HAs are formed at ppb levels during the cooking of muscle foods (Sugimura, 2000), and about 20 HAs have hitherto been identified (Felton et al., 2000). Naturally occurring substances present in meat—creatin/in/e (denotes creatine and creatinine), free amino acids and sugars—have been suggested as precursors of the imidazoquinoline or imidazoquinoxaline type (IQ-type) of HAs (for reviews see Jägerstad et al., 1998; Skog et al., 1998). Non-IQ-type HAs are believed to be produced by pyrolysis of amino acids and proteins (Tsuda et al., 1985). 2-Amino-3,4-dimethylimidazo[4,5-*f*]quinoline (MeIQ), MeIQx and PhIP have been classified as possible human carcinogens, and 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) as a probable human carcinogen (IARC, 1993). Classification is based on the results of long-term animal feeding studies in mice, rats and non-human primates (Adamson et al., 1990, 1994; Ohgaki et al., 1991; Wakabayashi et al., 1992). Recently reported data suggest that PhIP plays a significant role in human carcinogenesis (Nagao, 1999). Thus, the hypothesis that HAs in cooked foods may induce human cancer cannot be rejected.

Poultry is one of the most important protein-rich muscle food sources available today, and production and consumption are increasing rapidly. For example, between 1984 and 1994 the production of poultry

Abbreviations: AαC, 2-amino-9*H*-pyrido[2,3-*b*]indole; 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; HA, heterocyclic amine; harman, 1-methyl-9*H*-pyrido[3,4-*b*]indole; IQ, 2-amino-3-methylimidazo-[4,5-*f*]quinoline; IQx, 2-amino-3-methylimidazo[4,5-*f*]quinoxaline; MeAαC, 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole; MeIQ, 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline; MeIQx, 2-amino-3,8-dimethylimidazo-[4,5-*f*]quinoxaline; norharman, 9*H*-pyrido[3,4-*b*]indole; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; Trp-P-1, 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole; Trp-P-2, 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole.

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worldwide rose by 72%, while the production of pork, lamb and beef increased by only 41, 17 and 16%, respectively (Warriss, 2000). In Sweden, production and consumption of poultry increased by 25% between 1996 and 1999 (Jordbruksverket, 2000). In the United Kingdom, 37.5% of all the meat consumed consisted of poultry in 1995 (Warriss, 2000). The situation worldwide today is that more than one-quarter of meat consumed is poultry.

To date, more than 20 reports on HA content in poultry, mainly chicken, have been published. In many of these studies, severe cooking methods with temperatures well above 200°C (frying, grilling, broiling, barbecuing), or prolonged cooking times have been used (Sinha et al., 1995; Holder et al., 1997; Knize et al., 1997; Salmon et al., 1997). Since mutagenic/carcinogenic HAs may be produced under domestic cooking conditions, it is important to collect data on levels of HAs in normally cooked poultry to find means of minimising the intake of HAs.

The main objective of this study was to collect data on HA levels in various cooked chicken dishes, prepared under well-controlled cooking conditions representing normal domestic habits, and to estimate the variation in HA content in the same type of chicken prepared by different cooking methods. Another objective was to search for a correlation between surface colour and HA content. Taken together, this information could be used to estimate the intake of HAs from chicken dishes.

2. Materials and methods

2.1. Food samples

Frozen chicken breasts (sample I) were obtained from a slaughterhouse. Two different sorts of whole frozen chickens (II and III), frozen chicken breasts (IV) and chicken liver were purchased at local supermarkets. The frozen chicken breasts were properly thawed before cooking. The chicken liver was chopped before frying. Two barbecued chickens (A and B) were purchased at two different local supermarkets. Chicken bouillon concentrate (light brown liquid) and chicken stock cubes (light brown) were also purchased at local supermarkets.

2.2. Cooking methods

Chicken breasts (I) (weighing about 200 g) were prepared using several normal, domestic cooking conditions: boiling, deep-frying, pan-frying, oven-roasting, cooking in an unglazed clay pot or in a roasting bag in the oven, and oven broiling. For pan-frying, 10 g margarine was used for each sample, and the samples were turned once during frying. Pan residues were collected after some frying experiments. Before heating, thin chromium–aluminium thermocouples were inserted into the centre and just below the surface of the chicken breasts, and in the heating environment. The thermocouples were connected to a computer, and the temperature was recorded every 10 s. Whole chickens (II and III) were boiled in water for 4 h. Cooking methods, temperatures and times are shown in Table 1. The outer layer (1–3 mm) of the cooked samples was peeled off, and all cooked samples were lyophilised and stored at −18°C until analysis.

2.3. Analysis

Precursors (free amino acids, carnosine, creatin/in/e and glucose) in the uncooked products were analysed as described previously (Arvidsson et al., 1997).

The *weight loss* during cooking was determined by weighing.

The *colour* of the surface was measured in cooked chicken samples using a colorimeter (CR 200, Minolta Chroma Co., Ltd, Osaka, Japan). The difference in colour (ΔE) between a cooked sample and an uncooked (reference) sample was calculated using the expression:

$$\Delta E = [(L - L_{\text{reference}})^2 + (a - a_{\text{reference}})^2 + (b - b_{\text{reference}})^2]^{1/2},$$

where L corresponds to brightness, a defines the red component, and b the brown component (Calvo, 1996). Raw chicken breast was used as the reference.

HAs were analysed after solid-phase extraction using HPLC with UV and fluorescence detectors, as described by Gross and Grüter (1992), with some minor modifications (Solyakov et al., 1999). In addition, ethyl acetate was used instead of dichloromethane (Pais et al., 1999). To improve chromatographic efficiency, additional purification was carried out on some samples (Solyakov et al., 1999). A mixture of IQ, 2-amino-3-methylimidazo-[4,5-*f*]quinoxaline (IQx), MeIQ, MeIQx, 4,8-DiMeIQx, 2-amino-3,7,8-trimethylimidazo[4,5-*f*]quinoxaline (7,8-DiMeIQx), 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), harman and norharman was used as spiking solution (Solyakov et al., 1999). The amounts of HAs were corrected for incomplete recovery, and expressed in ng/g cooked sample.

3. Results

Common domestic cooking methods were used to prepare chicken, and MeIQx, 4,8-DiMeIQx, PhIP, harman and norharman (chemical structures are shown in Fig. 1) were identified in several samples. The results are summarised in Table 1.

Table 1
Cooking conditions, weight loss, colour and HAs in cooked chicken products

Chicken sample	Cooking conditions						HAs, ng/g[b]				
	n	Method	Temperature[a] (°C)	Time (min)	Weight loss (%)	Colour, (ΔE)	MeIQx	4,8-Di-MeIQx	PhIP	Harman	Norharman
I, breast	3	Boiling	100	23	22	4.5	nd	nd	nd	0.1[c]	nd
II, meat	3	Boiling	100	240			nd	nd	nd	0.2	0.4
II, bouillon	3	Boiling	100	240			nd	nd	nd	0.5±0.2[d]	0.4±0.2[d]
III, meat	3	Boiling	100	240			nd	nd	nd	0.2	0.4
III, bouillon	3	Boiling	100	240			nd	nd	nd	Trace	Trace
I, breast	5	Deep-frying	160	11	21	26.0	Trace	Trace	Trace	0.5[c]	0.3[c]
I, breast	5	Pan-frying	140	14	18	22.1	0.1	0.1	Trace	0.5±0.2[c]	0.3[c]
I, breast	3	Pan-frying	170	16	21	29.6	0.3	0.1	0.7±0.2	0.1	0.3
I, breast	3	Pan-frying	190	18	26	34.9	1.0	0.6±0.3	10.5±3.0	1.0±0.6	1.9±0.7
I, breast	3	Pan-frying	220	12	25	38.2	1.0±0.3	0.5	29.7±1.4	5.7±1.2	3.5±2.7
I, breast	5	Pan-frying	190	34	36	38.6	0.3±0.1	0.3	38.2±2.0	6.9±2.4[c]	7.5±2.8[c]
II, breast	5	Pan-frying	190	31	38	36.0	1.8±0.3	0.4±0.1	12.2±0.4	1.8±0.7[c]	3.4±0.3[c]
III, breast	5	Pan-frying	190	31	38	38.8	1.7±0.4	0.4	19.3±0.1	3.1±0.5[c]	4.0±1.8[c]
IV, breast	2	Pan-frying	170	20	20		0.2	0.1	nd	0.8[c]	1.0±0.2[c]
IV, breast	3	Pan-frying	220	20	29		1.5±0.2	0.4	1.8±0.1[c]	2.6±0.1[c]	5.1±0.4[c]
I, breast, pan residues	3	Pan-frying	170	16			nd	nd	Trace	Trace	Trace
I, breast, pan residues	3	Pan-frying	200	18			nd	nd	0.1	Trace	0.1
I, breast, pan residues	3	Pan-frying	220	12			nd	nd	0.2	Trace	0.1
I, liver	3	Pan-frying	190	9			nd	nd	nd	nd	nd
I, breast	5	Roasting	175	25	12	6.5	nd	nd	nd	Trace	Trace
I, breast	3	Roasting	245	40	40		1.7±0.2	0.3±0.1	3.0±0.5[c]	3.3±0.2[c]	1.7±0.4[c]
I, breast OVEN	3	Clay pot	200	25	9	2.4	nd	nd	nd	0.1[c]	nd
I, breast	3	Roasting bag	200	25	22	1.3	nd	nd	nd	0.1[c]	0.1[c]
I, breast	5	Broiling	200	38	16	6.9	nd	nd	0.1	Trace	Trace
A, meat	3	Barbecuing, com.					Trace	nd	nd	1.5±0.2[c]	1.5±0.1[c]
A, skin	3	Barbecuing, com.					1.1±0.2	0.2	nd	6.5±0.4[c]	10.3±0.1[c]
B, meat	3	Barbecuing, com.					Trace	nd	nd	1.8±0.2[c]	1.5±0.3[c]
B, skin	3	Barbecuing, com.					2.3±0.4	1.0±0.1	0.8±0.2[c]	9.6±1.6[c]	12.9±0.4[c]
Bouillon concentrate	3						0.1[d]	nd	nd	8.4±2.0[cd]	8.0±1.7[cd]
Bouillon cube	3						nd	nd	nd	4.6±0.2[c]	3.2±2.3

nd = not detected; com. = commercial.

[a] Denotes the temperature in the surrounding liquid, frying pan, or in the oven.

[b] Mean value±standard deviation; if SD <0.1 ng/g, the values are not given.

[c] Fluorescence data (the identity was confirmed with UV spectra).

[d] ng/ml.

In *boiled* chicken meat and bouillon, only harman and norharman were detected, at levels up to 0.5 ng/g cooked product. In *deep-fried* chicken breast, harman and norharman were detected, at similar levels, together with traces of MeIQx, 4,8-DiMeIQx and PhIP. In *pan-fried* chicken breast, MeIQx, 4,8-DiMeIQx, PhIP, harman and norharman were detected in most of the samples; the amount of MeIQx was below 2 ng/g, and 4,8-DiMeIQx below 1 ng/g, while the amount of PhIP ranged from non-detectable to almost 40 ng/g. The amounts of harman and norharman were less than 10 ng/g each. In the corresponding pan residues, MeIQx was not detected but low amounts of PhIP, trace–0.2 ng/g. No HAs were detected in the pan-fried chicken liver. In samples prepared in the *oven*, one roasted sample contained MeIQx, 4,8-DiMeIQx, PhIP, harman and norharman, while the amounts of HAs were below 0.1 ng/g for the other samples.

The *commercially barbecued* chicken *meat* contained traces of MeIQx, together with harman and norharman. The chicken *skin* contained MeIQx, 4,8-DiMeIQx and PhIP in amounts up to 2.3 ng/g, and harman and norharman in amounts up to around 13 ng/g. The commercial bouillon concentrate contained low amounts of MeIQx, and both the concentrate and the stock cube contained harman and norharman.

IQ, IQx, MeIQ, Trp-P-1, Trp-P-2, AαC and MeAαC were not detected in any of our samples. Extraction recovery rates varied depending on the meat matrix and cooking conditions, and were 73–91% for MeIQx, 51–88% for DiMeIQx, 61–100% for PhIP, 48–80% for harman and 52–75% for norharman.

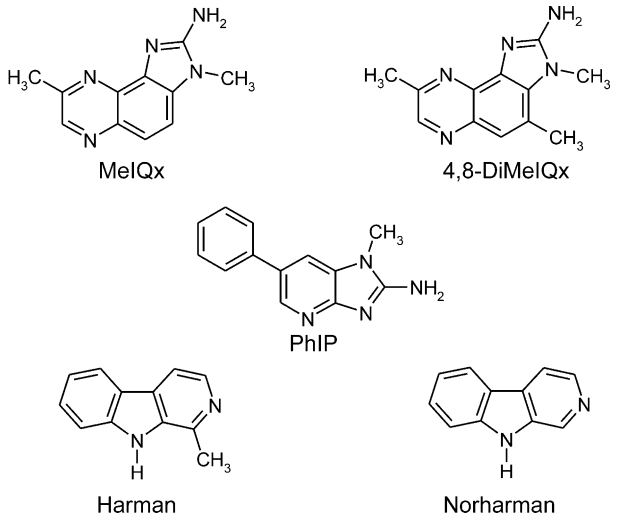

Fig. 1. Chemical structures of the HAs detected in cooked chicken samples.

3.1. Colour

The surface colour (ΔE) of some samples was measured, see Table 1. The colour intensity increased with increasing temperature during pan-frying. Furthermore, the colour of deep-fried and pan-fried samples were found to be within the same range, which differed from that of boiled or oven-cooked samples.

3.2. Precursor composition in different chicken breasts

To study the influence of precursor composition on the amount of HAs, chicken breasts I, and the breasts from whole chickens II and III, were analysed for free amino acids, creatin/in/e and glucose. The results expressed in μmol/g wet weight are shown in Table 2. The total amount of free amino acids was about 32 μmol/g in sample I, and about 25 μmol/g in samples II and III. The three amino acids present in the highest amounts were tryptophan, alanine and glutamic acid. The content of creatin/in/e varied between 24 and 28 μmol/g, and the ratio between creatine and creatinine was about 10:1. The glucose contents differed considerably and were about 8, 15 and 27 μmol/g for samples I, II and III, respectively.

Table 2
HA precursors (μmol/g wet tissue) in different chicken breasts, $n = 2$

Compound	Sample I	Sample II	Sample III
Creatin/in/e[a]	27	28	24
Glucose ($n = 4$)	7.7	15.1	26.7
Carnosine	6.1	5.2	5.3
Free amino acids (total)	32.2	24.7	25.3
Taurine	0.25	1.00	1.40
Aspartic acid	1.83	1.25	1.02
Threonine	1.54	1.12	1.11
Serine	2.29	1.66	1.78
Aspargine	nd	nd	nd
Glutamic acid	3.10	2.44	2.38
Glutamine	1.27	0.60	0.99
Proline	1.50	1.21	0.65
Glycine	2.05	1.68	1.39
Alanine	3.14	2.58	2.50
Valine	1.46	1.18	1.16
Cystein	nd	nd	nd
Methionine	0.64	0.48	0.43
Isoleucine	0.96	0.72	0.61
Leucine	1.71	1.44	1.48
Tyrosine	0.84	0.59	0.66
Phenylalanine	0.69	0.57	0.61
Ornithine	Trace	0.08	Trace
Lysine	2.25	1.46	1.30
Histidine	1.09	0.78	0.64
Tryptophan	4.34	3.05	3.60
Arginine	1.28	0.78	1.82

[a] The ratio creatine:creatinine was about 10:1.

4. Discussion

The boiling of meat products (pig, beef, chicken) for several hours to obtain a gelatinous structure of the final meat dish (aspic or galantine) is a common practise in Russia. Two varieties of chicken were boiled for 4 h, and harman and norharman were detected both in meat and bouillon. To our knowledge, this is the first report on analysis of HAs in boiled chicken. In another study, no HAs were detected in stewed chicken; however, the samples were not tested for harman or norharman (Sinha et al., 1995).

Deep-frying of chicken breasts produced traces of MeIQx, 4,8-DiMeIQx and PhIP, and harman and norharman in amounts below 1 ng/g. In another study, up to 12 different HAs were found in amounts up to about 3 ng/g in chicken legs deep-fried at 100–200°C for 5–15 min (Chiu et al., 1998).

Pan-frying produced MeIQx in amounts below 2 ng/g, which is in accordance with most literature data. However, there is one report of higher amounts of MeIQx, 10.4 ng/g, in chicken breasts pan fried for 15 min at 220°C (Krul et al., 2000). In our study, both MeIQx and PhIP were in general identified in the same samples; PhIP being found at higher levels than MeIQx. In the pan-fried samples, the amount of PhIP increased with increasing cooking temperature, from below 1 ng/g up to 38.2 ng/g. Our data are in the same range as those found in the literature, with some exceptions: for example, 70 ng/g PhIP was detected in pan-fried, skinless, boneless chicken breasts (Sinha et al., 1995). PhIP seems to form easily at higher temperatures. When chicken breasts were pan-fried at 190°C for 18 min or at 220°C for 12 min, the weight losses were similar, about 25%, and the contents of MeIQx and 4,8-DiMeIQx were similar in the two samples, but, interestingly, the content of PhIP was about three times higher in the sample cooked at the higher temperature. This observation

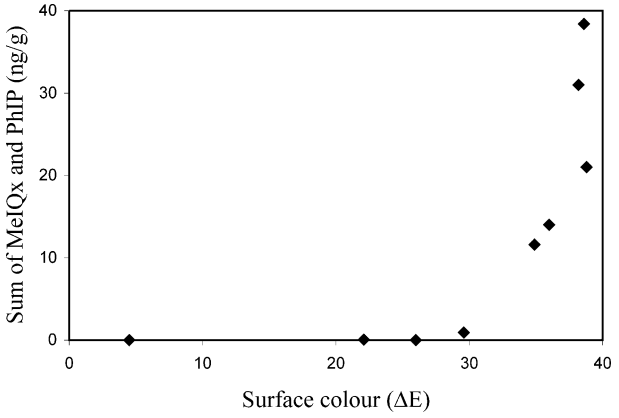

Fig. 2. Content of MeIQx and PhIP in chicken breasts in relation to surface colour development.

clearly indicates that controlling cooking temperature is a way of minimising the formation of HAs.

Oven cooking yielded in general non-detectable amounts of MeIQx and PhIP, with one exception: roasting at 245°C for 40 min produced MeIQx (2 ng/g) and PhIP (3 ng/g). Skog et al. (1997), reported the presence of MeIQx (nd–0.02 ng/g), 4,8-DiMeIQx (nd–0.01 ng/g) and PhIP (0.04–0.6 ng/g) in oven roasted (150 and 200°C) chicken breasts. Cooking chicken in an oven (roasting/broiling, cooking in a clay pot or a roasting bag) at temperatures at or below 200°C, is another way of minimising HA formation.

An interesting observation was made from the analysis of the commercially barbecued chickens. The meat and skin were analysed separately, and MeIQx and 4,8-DiMeIQx were detected in the skin from sample A, while only traces of MeIQx were detected in the meat. The skin from sample B contained MeIQx, 4,8-DiMeIQx and PhIP, but these compounds were not detected in the meat. The low level of HAs agrees well with results from another study, where no HAs were detected in fast-food chicken samples (Knize et al., 1995). The presence of HAs in the skin can be explained by direct exposure to the heat source, while the skin acts as an insulating layer for the meat. To the best of our knowledge, HAs have not earlier been reported in skin from barbecued chicken. Estimated amounts of HAs in an ordinary portion size, that is, half a chicken (250 g meat + 35 g skin), are shown in Table 3. From these data, it is obvious that the intake of HAs from grilled chicken can be decreased by not consuming the skin.

No HAs were detected in the fried chicken liver sample. This is in agreement with results from earlier experiments, where mutagenic activity was not detected in fried minced liver (Laser Reuterswärd et al., 1987). This can be explained by the very low level of creatin/in/e detected in raw chicken liver, 2 μmol/g wet weight, which was less than 1/10 of the amount in chicken meat. Creatine is a key precursor of IQ-type of HAs and PhIP (Jägerstad et al., 1983, 1998; Knize et al., 1988; Skog et al., 1998).

Table 3
Total amount of HAs in an ordinary portion of commercially barbecued chicken, ng/portion (half a chicken)

Sample	Mutagenic HAs (MeIQx, 4,8-DiMeIQx, PhIP)	Co-mutagenic HAs (harman, norharman)
A		
Meat	<3	750
Skin	46	590
B		
Meat	<3	830
Skin	140	790

Two different samples of commercial chicken flavours were analysed. MeIQx was detected in the bouillon concentrate, while other mutagenic HAs were not detected at all. Ppb levels of HAs in in chicken flavour paste other types of bouillon concentrate have been reported earlier (Fay et al., 1997; Solyakov et al., 1999).

Harman and norharman were detected in almost all samples, at levels below 15 ng/g. This agrees well with literature data. Harman and norharman are often referred to as co-mutagens, because they are not mutagenic in the Ames/*Salmonella* test, but enhance the mutagenic activity of other compounds; for example, norharman enhances the mutagenic effects of Trp-P-1 and Trp-P-2 (Sugimura et al., 1982). Furthermore, harman and norharman have been discussed in relation to neurotoxins and enzyme inhibitors (de Meester, 1995; Kuhn et al., 1996). Thus, it is of great importance to analyse the amounts of harman and norharman in cooked foods.

To study the influence of precursor composition on HA formation, three different chicken breasts (I, II and III) were pan-fried at 190°C for 31–34 min. Sample I contained more than twice the level of PhIP (38.2 ng/g) as the other two samples. Model experiments have shown that phenylalanine and creatine are precursors of PhIP (Felton and Knize, 1991), and that PhIP is produced from leucine, isoleucine and tyrosine when heated together with creatine, with or without sugars (Övervik et al., 1989; Johansson et al., 1995). In our study there was no significant difference between the content of these amino acids in the uncooked samples. However, sample I, with the highest level of PhIP, contained the lowest amount of glucose, which indicates that a high concentration of glucose reduces PhIP formation. Model experiments have shown that MeIQx is formed from many different amino acids (Johansson et al., 1995), and that the formation of MeIQx increases with increasing glucose concentration up to about half of the molar amount of creatin/in/e and free amino acids, probably due to the involvement of the Maillard reaction (Skog and Jägerstad, 1991). The approximate ratio between glucose, creatine and amino acids in sample I was 3.5:1:1, in sample II 2:1:1, and in sample III 1:1:1, and perhaps the ratio in sample I (glucose concentration

higher than the concentration mentioned above) was less favourable for MeIQx formation. These results show that not only the cooking conditions, but also meat composition influence the formation of HAs.

In an attempt to examine the variation in HA formation during domestic cooking, chicken breasts (sample IV) were fried for 20 min at low and high settings (corresponding to about 170 and 220°C). The amount of MeIQx increased by a factor 6, and PhIP increased from non-detectable amounts up to 1.8 ng/g, comparing the low-temperature and high-temperature cooking methods. These results give an indication of the wide range of HA content in home-cooked chicken breasts.

Comparing the colour measurements (ΔE) in Table 1, it is obvious that samples cooked in close contact with a fat layer differed most from the reference sample. Furthermore, data from the pan-fried samples (140–220°C) indicate that colour intensity increases with increasing temperature, as does the amount of HAs formed. Chicken breasts (I, II and III) pan-fried at 190 and 220°C have similar ΔE values, but the amounts of MeIQx and especially PhIP differ markedly. Fig. 2 shows the amount of MeIQx and PhIP in relation to colour. For ΔE values lower than 30, only low amounts of HAs are formed. For ΔE values higher than 30, no correlation with HA formation can be seen. Thus, our results clearly show that it is not possible to estimate the content of HAs from colour measurements alone. In some assessments of human exposure to HAs, photographs showing varying surface colour have been used to estimate the degree of doneness and indirectly HA content (Augustsson et al., 1997, 1999; Sinha and Rothman, 1997).

In conclusion, our results show that the content of HAs in cooked chicken is low if prepared at low temperatures, and that the formation of HAs increases with increasing cooking temperature. PhIP formation seems to start accelerating at pan-frying temperatures around or above 200C. Thus, controlling temperature is important in minimising the formation of HAs. The intake of HAs from barbecued chicken can be reduced by not consuming the skin. Colour development increases with cooking temperature, but no correlation could be found with HA content. Our results on HA content in cooked chicken, prepared under various but well-controlled cooking conditions, may be used in combination with dietary assessments to estimate the exposure to HAs from chicken.

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