DOI 10.1002/mnfr.200400024 Mol. Nutr. Food Res. 2004, *48*, 292 – 298

Heterocyclic amines in some Swedish cooked foods industrially prepared or from fast food outlets and restaurants

Eva Borgen and Kerstin Skog

Division of Applied Nutrition and Food Chemistry, Department of Food Technology, Engineering and Nutrition, Lund University, Lund, Sweden

Accurate assessment of human intake of mutagenic/carcinogenic heterocyclic amines (HAs) is necessary for epidemiological studies and future risk assessment. Using questionnaires, the frequency of consumption of specific dishes can be obtained at an individual level and linked to analyzed concentrations of different compounds in corresponding dishes. Some typical Swedish cooked meat dishes, hamburgers and kebab, industrially prepared or from fast food outlets and restaurants, were analyzed regarding their content of 11 different HAs. The amount of each of these compounds was below 0.1 ng/g cooked weight in most of the industrially prepared products. The total amount of HAs was highest in the kebab samples. The intake of HAs from 200 g of the dishes was estimated to range from not detectable levels to 0.6 μg. The results of the present study indicate that the content of HAs in a specific dish may vary with origin, and that the concentrations of HAs in commercial fried meat products are generally low, although some of these food items may contain elevated amounts.

Keywords: Fast food / Food mutagens / Hamburgers / Heterocyclic amines

Received: February 2, 2004; revised: April 26, 2004; accepted: April 27, 2004

1 Introduction

Eating habits are changing in the Western world and there is an increased tendency to rely on foods produced by the food industry, restaurants, and fast food chains. In a survey of eating habits in Sweden, only about 40% of those interviewed claimed that they ate all their hot meals at home [1]. In another Swedish survey of eating habits, it was found that about 60% of those who ate lunch at restaurants did so on a daily basis [2].

Heterocyclic amines (HAs) belong to a group of mutagenic/carcinogenic compounds formed during cooking of foods, especially muscle meat [3]. Since some HAs are considered to be possible human carcinogens [4], much work has been devoted to clarifying their role in the etiology of cancer. Several epidemiological studies have shown a high meat consumption to be associated with an increased risk for cancer, and it has been suggested that HAs present in cooked meat and fish may explain some of the effect (for a review see [5]). However, other studies have shown conflicting results (for a review see [6]).

In order to make reliable risk estimates, there is a need for information on the intake of HAs, and such information may be obtained from data on levels of HAs in cooked foods combined with data on dietary habits. The chemical structures of some of the compounds are shown in Fig. 1. As food preparation practices and dietary habits differ among countries, it is important to obtain data on the amounts of HAs in commonly eaten foods in various countries. The amounts of HAs in cooked meat vary with cooking conditions, meat type, and shape of the product (for a review see [7]), and may be influenced by the recipes, for example, the addition of ingredients such as potato starch,

Correspondence: Kerstin Skog, Division of Applied Nutrition and Food Chemistry, Department of Food Technology, Engineering and Nutrition, Lund University, PO Box 124, SE-221 00 Lund, Sweden
E-mail: Kerstin.Skog@inl.lth.se
Fax: + 46-46-222-4532

Abbreviations: IQ, 2-amino-3-methylimidazo[4,5-*f*]quinoline, CAS no. 76180-96-6; **MeIQ**, 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline, CAS no. 77094-11-2; **IQx**, 2-amino-3-methylimidazo[4,5-*f*]quinoxaline, CAS no. 108354-47-8; **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; **Aα**C, 2-amino-9*H*-pyrido[2,3-*b*]indole, CAS no. 26148-68-5; **MeAα**C, 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole, CAS no. 68006-83-7; **Norharman**, 9*H*-pyrido[3,4-*b*]indole, CAS no. 244-63-3; **Harman**, 1-methyl-9*H*-pyrido[3,4-*b*]indole, CAS no. 486-84-0; **Trp-P-2**, 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole, CAS no. 62450-10-3; **Trp-P-1**, 3-amino-1,4-dimethyl-5*H*-pyrido-[4,3-*b*]indole, CAS no. 62450-06-0

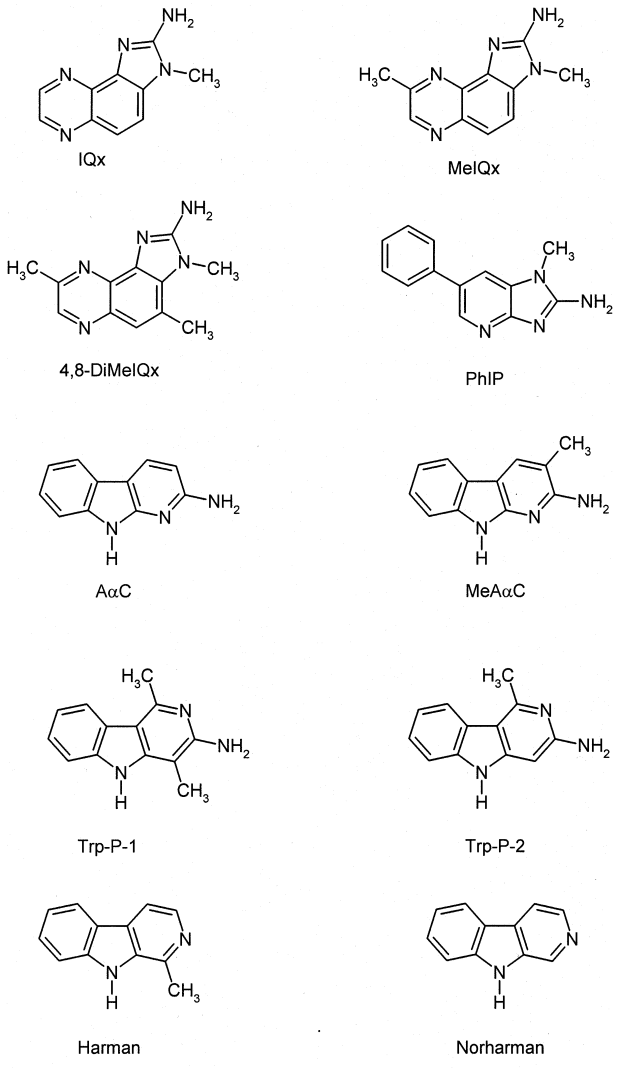

Figure 1. Chemical structures and trivial names for some HAs.

common salt, sugar, and spices [8 – 12]. Marinades, brine or seasoning that contain antioxidants, may reduce the formation of HAs [10, 13 – 15].

There are a great number of reports on HAs in foods prepared in the laboratory, *i.e.*, in studies aimed at investigating the influence of different cooking conditions on the formation of HAs (for a review see [7]). Less has been reported on the content of HAs in foods from restaurants, fast food outlets or ready-to-eat meals manufactured by the food industry [16 – 22]. The objective of this study was to estimate the content of HAs in some commonly eaten fried meat dishes from fast food outlets, restaurants and in industrially prepared foods ready for frying or reheating. These data can be used together with information on dietary habits in future risk assessments of HAs.

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 groups of HAs were used as reference compounds: (i) IQx compounds: 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), (ii) PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine), and (iii) aminocarbolines: Trp-P-1 (3-amino-1,4-dimethyl-5H-pyrido[4,3-*b*]indole), Trp-P-2 (3-amino-1-methyl-5H-pyrido[4,3-*b*]indole), Norharman (9*H*-pyrido[3,4-*b*]indole), Harman (1-methyl-9*H*-pyrido[3,4-*b*]indole), AαC (2-amino-9H-pyrido[2,3-*b*]indole), and MeAαC (2-amino-3-methyl-9H-pyrido[2,3-*b*]indole), and were purchased from Toronto Research Chemicals (Toronto, Canada). The chemical purity of the synthetic references was higher than 99%, according to the manufacturers. A mixture of the different HAs dissolved in methanol (2 ng of each compound/μL) was used as a spiking mixture. Materials for solid-phase extraction, diatomaceous earth, propylsulphonic acid silica (PRS) and C_{18} columns (Isolute) were obtained from Sorbent AB (Västra Frölunda, Sweden).

2.2 Food samples

Kebab samples were obtained from two fast food outlets. Hamburgers were obtained from a local lunch restaurant and three fast food outlets. Ground meat and industrially manufactured hamburgers and meat rolls intended for reheating or frying were purchased from local supermarkets. The meat products were categorized into three different groups: (A) products from a lunch restaurant or fast food outlet, (B) cooked products intended for reheating, and (C) ready-made or home-made products fried at home. Specifications of the products and degree of cooking are summarized in Table 1. The degree of cooking of the samples was judged by the colors of the surface and of the center of the products, and was considered to be "medium done" except for four samples that were considered to be "well done".

2.3 Sample preparation

The crust of the fried products (1–2 mm of the outer layer) was removed using a scalpel, except for the kebab samples that were very thin. All samples were freeze-dried and

Table 1. Specification of the food products, cooking method, and level of cooking

Food product	Obtained from	Ingredients	Cooking method	Level of cooking
A1 Hamburger	Fast food chain	Beef (100%)	Grilled	Medium
A2 Hamburger	Fast food chain	Beef (80%), potato, water, potato flour, common salt, seasoning, potato fiber, sodium phosphate, and potassium phosphate	Fried	Medium
A3 Hamburger	Fast food chain	Beef (100%), common salt, and pepper	Fried	Well done
A4 Hamburger	Lunch restaurant, in-house made	Beef (100%), common salt, and pepper	Fried	Well done
A5 Kebab	Fast food chain	Beef	Fried	Medium
A6 Kebab	Fast food chain	Beef	Fried	Medium
B7 Meat patty	Supermarket	Pork and beef (total meat content 67%), potato, potato flour, glucose, salt, potato fiber, seasoning, onion, yeast extract, sugar, potassium glutamate, aroma compounds, ascorbic acid	Industrially fried, chilled, reheated	Medium
B8 Meat rolls	Supermarket	Pork and beef (total meat content 68%), potato, water, potato flour, common salt, glucose, potato fiber, yeast extract, seasoning, onion, and garlic	Industrially fried, chilled	Well done
C9 Hamburger	Ground meat from supermarket	Beef (100%), common salt, and pepper	Fried at home	Medium
C10 Hamburger	Supermarket	Beef (89%), water, egg, whey powder, potato flour, common salt, seasoning, and ascorbic acid	Industrially prepared, frozen, fried at home for 5 or 8 min	Medium and well-done

stored at –18°C until analyzed. The samples were extracted and purified according to the solid-phase extraction method of Gross and Grüter [23] with some modifications [24]. Briefly, the samples were homogenized in 1 M NaOH and mixed with diatomaceous earth. Many of the products had a high fat content and to avoid problems associated with clogged columns, the NaOH solution was saturated with NaCl. Ethyl acetate was used as the extraction solvent. The method yielded two fractions, one containing IQx compounds and one containing PhIP and aminocarbolines. Extraction recovery rates for the different HAs were determined by the addition of 100 µL spiking mixture to one sample extracted in parallel with the unspiked samples. For each product, two or three samples were extracted and analyzed.

2.4 Identification and quantification of HAs

In a first set of experiments, the samples were analyzed by HPLC (Varian 9010; Harbor City, CA, USA) using a photodiode array UV detector (Varian 9065, Polychrome) or a programmable fluorescence detector (Varian LC 9070) [25]. As the amounts of HAs in the cooked food samples were low, three purified extracts containing similar samples were pooled together. However, the complex sample matrix contained interfering co-eluting compounds which often made accurate quantification impossible. Thus, in the next set of experiments, the samples were analyzed using HPLC (Spectra-Physics P2000; San José, CA, USA) combined with a mass spectrometer (LCQ_{DECA} ion-trap; ThermoFinnigan, San José, CA, USA) as earlier described [26]. This method was also used in an intercalibration study on determination of HAs in food products [27]. The chromatographic conditions were: solvent A – water (pH adjusted to 3.5 with acetic acid)/acetonitrile, 95/5, and solvent B – water (pH adjusted to 3.5 with acetic acid)/acetonitrile, 5/95. The effluent from the column was analyzed using mass spectrometry with an electrospray interface. The heated capillary temperature was set at 325°C. Nitrogen was used as sheath gas and auxiliary gas. The MS-detector was operated in positive mode. Analyses were achieved in selected ion monitoring (SIM) mode. The HAs were identified and quantified with Xcalibur software (ThermoQuest, San José, CA, USA), using retention times and mass spectra from reference samples of known concentrations, run under the same conditions.

3 Results and discussion

3.1 Common dishes and cooking methods

A pilot study was performed to establish the most commonly served meat dishes in lunch restaurants. A small group of chefs (13) was asked to report the five most commonly served meat dishes and the corresponding cooking methods. Chicken was the most common dish, followed by meatballs, loin of pork, "Falun sausage" and meat patties. Oven roasting was the most common cooking method, however, meat patties were usually fried. Some of the chefs reported that they first fried the meat to obtain an appetizing

surface and that it was then cooked through in the oven. Studies of dietary habits in Sweden have shown that minced meat dishes are among the most commonly eaten dishes [1, 2, 28]. In a small survey on dietary habits in Sweden, frying was reported to be the most commonly used cooking method: 61% of the hot meat meals were fried on weekdays and 54% on Sundays, and 55% of the main dishes included meat on weekdays and 66% on Sundays [1]. In another survey of dietary habits in Sweden, it was found that of those who ate lunch at restaurants, one out of four chose to eat a dish containing a piece of whole meat; other common dishes included minced meat or sautéed meat [2]. Based on these data, we decided to investigate the content of HAs in various fried minced meat products.

3.2 Identification of HAs and extraction recoveries

In the first set of experiments, the identities of MeIQx, Trp-P-1, AαC, and MeAαC in the samples were confirmed by on-line recorded UV spectra of the compounds together with reference spectra. The sample matrix was, however, very complex and the chromatograms showed many peaks originating from interfering compounds. This prompted us to analyze the samples using LC-MS. Generally, HAs detected with the UV detector were also detected with MS and good agreement was found between the concentrations of HAs determined with the two methods. Some HAs were more frequently found with MS than with UV detection which implies that equipment with low detection limits, for example, LC-MS, is useful when analyzing low levels of HAs. Another way of improving the analysis using UV detection is to reduce the amounts of interfering substances by the addition of an extra purification step [29], but the recovery may be somewhat reduced.

The extraction recoveries varied with the compound and, to some extent, also with the food product. Average recoveries varied from 40 to 70% for the IQx compounds, 55 to 100% for PhIP, and for the aminocarbolines the recovery varied from 20 to 80%. When the recovery of HAs was investigated using different commercial brands of solid-phase extraction cartridges [30], the highest variation in the recovery was observed for Trp-P-1 and Trp-P-2, ranging from 3 to almost to 60%, and the detection limits were estimated to be 0.002 ng/g for most of the HAs. Using LC-MS in our study, the limit of quantification was estimated to be 0.01 ng/g cooked product but sometimes higher depending on the sample matrix.

3.3 Concentrations of HAs

Using LC-MS, HAs were found in all samples but one and the results, corrected for incomplete recovery, are summarized in Table 2. The amounts are calculated as ng per g cooked food product and are given as the average of two determinations. The difference between duplicate determinations was generally below 20%. Using only the outer layer of the food samples increased the detection sensitivity, however, the amounts of HAs may be slightly underestimated due to the presence of low amounts of HAs in the inner parts of the products [32, 33]. In earlier studies, the amounts in the inner parts ranged from not detectable up to 15–30% of the total of MeIQx and PhIP (for samples fried at the highest temperatures) [32].

The concentrations of HAs ranged from not detectable levels up to 0.40 ng/g for the IQx compounds, 0.43 ng/g for PhIP and 0.75 ng/g for the aminocarbolines. Our data are in the same range as those in the literature, from other studies on HAs in minced meat products from restaurants, fast food outlets or industrial sources, see Table 3 [16–22, 30]. For example, MeIQx has been reported to range from not detectable up to 1.8 ng/g and PhIP from not detectable up to 18.4 ng/g [16]. Interestingly, Trp-P-1, Trp-P-2, AαC, and MeAαC were detected in several samples in amounts up to 0.69 ng/g cooked weight. To our knowledge, the presence of Trp-P-1, Trp-P-2, AαC, and MeAαC has not previously been reported in this type of commercially cooked food, but in commercial meat extracts [24]. Furthermore, data on these aminocarbolines in laboratory-cooked minced meat products are scarce but in general, the concentrations are in agreement with our data (for a review, see [3]).

3.4 Various recipes and modes of cooking

The total amount of HAs in industrially fried food (meat patty and meat roll) was lower than in fried food from the restaurant, fast food outlets, and in the meat dishes cooked at home. A possible explanation for this may be the use of different and well-controlled cooking conditions in industry where the meat is usually heated for a short time to obtain a crust, typically by contact frying or deep-fat frying, and is then cooked through in an oven. Frying is a common method of cooking in restaurants and fast food outlets, as well as in homes where cooking time and temperature may be less well controlled. The highest levels of HAs were found in the two kebab samples; probably the heating conditions had been more extreme than for the other food samples. The influence of cooking time is illustrated by an experiment where prefabricated hamburgers were fried for either 5 min according to the instructions on the package or for 8 min. The amounts of IQx compounds and PhIP in the 5 min sample were below 0.01 ng/g, while the 8 min sample contained up to 0.14 ng/g. These results indicate that controlling the cooking time is of importance for keeping the HA formation at a minimum level. Some of the tested products contained other ingredients such as common salt,

Table 2. Amounts of HAs (ng/g cooked weight) in cooked meat products analyzed using LC-MS

	Hamburger				Kebab		Meat roll	Meat patty	Hamburger		
HA	A1	A2	A3	A4	A5	A6	B7	B8	C9	C10 (5 min)	C10 (8 min)
IQx	0.02	0.05	0.14	–[b)]	0.16	0.40	–	–	–	<0.01	0.14
MeIQx	0.02	0.06	0.18	0.02[c)]	0.14	0.24	nd	nd	0.17	<0.01	<0.01
7,8-DiMeIQx	0.02	nd[a)]	nd	–	0.16	0.16	–	–	–	<0.01	0.04
4,8-DiMeIQx	0.03	0.01	nd	–	0.22	0.09	–	–	–	<0.01	0.05
PhIP	0.06	0.04	0.03	D[d)]	0.22	0.25	nd	0.03	0.43	0.02	0.05
NH	0.13	nd	0.52	–	nd	0.71	–	–	nd	–	–
H	0.01	nd	0.02	–	0.75	nd	–	–	nd	–	–
Trp-P-2	0.13	0.04	0.35	0.08	0.45	0.69	nd	0.10	0.21	0.06	–
Trp-P-1	nd	nd	0.62	0.09	nd	nd	nd	0.11	0.29	0.09	–
AαC	0.02	0.03	0.09	nd	0.52	0.12	nd	nd	nd	nd	–
MeAαC	0.02	0.01	0.05	nd	0.16	0.06	nd	0.02	nd	nd	–
Total	0.46	0.24	2.00	0.19	2.78	2.72	nd	0.26	1.10	0.17	0.28

a) nd, not detected
b) Not analyzed
c) UV-detection
d) Detected, but the presence not confirmed with UV
Values are the mean of duplicate determinations and are corrected for incomplete recovery.
A, lunch restaurant or fast food outlet; B, cooked products intended for reheating; C, ready-made or home-made products fried at home

Table 3. Literature data on contents of HAs (ng/g) in commercially cooked ground meat products

Food type	IQ	MeIQ	MeIQx	DiMeIQx	PhIP	Ref.
Hamburger			0.2–1.8	nd–0.1	1.8–18.4	[16]
Hamburger			1.0–1.0	0.3–0.2	0.5–1.8	[17]
Ground beef			nd	nd	nd	[18]
Beefburger	0.1	0.4	0.4	0.1	nd	[19]
Ground beef			0.26–0.68	0.1–0.28	nd	[20]
Ground beef			<0.1–0.3	nd–0.1	0.1–0.6	[21]
Ground beef	nd	nd	nd	nd	0.3	[22]
Meatballs	0.2	0.3	0.7	0.2	0.6	[19]

sodium phosphate, potato, potato flour, and/or potato fibers. These ingredients confer a better water-holding capacity, thus reducing the transport of precursors towards the surface during cooking [8]. This may explain the low amounts of HAs in these products.

3.5 Intake

The concentrations of HAs in the cooked meat dishes in our study were used to indirectly assess the intake from eating 200 g of the food items studied. These calculations resulted in estimates of the total intake of HAs of less than 0.6 μg. This is in agreement with results from another study on the content of HAs in Swedish foods in which the daily intake of HAs was estimated to be 0.003–0.94 μg per person [19]. Other studies on the intake of HAs based on concentrations in cooked food have revealed levels varying from 0.04 to 8.4 μg/day per person (for a review, see [5]).

4 Concluding remarks

For epidemiological studies and future risk assessment it is necessary to increase our knowledge on exposure levels to HAs. Accurate assessment of the human intake of HAs requires data on these compounds in common cooked foods. Our study shows that the concentrations of HAs in commercial fried meat products are generally low, but that some food items may contain elevated amounts. Taken together, our data show that ingredients and cooking conditions can influence the concentration of HAs in the cooked products.

We thank MSc P. Brglez and MSc T. Cucu for excellent technical assistance. This work was supported by the Swedish Council for Forestry and Agricultural Research, The Dir. A. Påhlsson Foundation, The Royal Swedish Society of Science and was also carried out with financial support from the Commission of the European Community's specific RTD programme, "Quality of Life and Management of Living

Resources", QLK1-CT99-001197, "Heterocyclic Amines in Cooked Foods – Role in Human Health". It does not necessarily reflect Commission's views and in no way anticipates the Commission's future policy in this area.

5 References

[1] Kjärnes, U., Eating Patterns – a Day in the Lives of Nordic People. *SIFO Report No. 7. Statens Institutt for forbruksforskning* 2001, *1*, 75 – 77.

[2] Westman, C., Skans, M., Val av Lunchrätt. En orienterande studie för Livsmedelsverket. Project number 3615210, version 2. 2001, p. 17 (in Swedish).

[3] Felton, J., Jägerstad, M., Knize, M., Skog, K., Wakabayashi, K., Contents in Foods, Bevarages and Tobacco. In: Nagao, M., Sugimura, T. (Eds.), *Food borne Carcinogens, Heterocyclic Amines*, John Wiley & Sons, Chichester, New York 2000, pp. 332 – 347.

[4] International Agency for Research on Cancer, Some Natural Occurring and Synthetic Food Components, Furocoumarins and Ultraviolet Radiation. *IARC Monographs of the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Monograph*, Lyon 1993, *56*, 165 – 195.

[5] Augustsson, K., Steineck, G., Cancer Risk Based on Epidemiological Studies. In: Nagao, M., Sugimura, T. (Eds.), *Food borne Carcinogens, Heterocyclic Amines*, John Wiley & Sons, Chichester, New York 2000, pp. 332 – 347.

[6] Norat, T., Riboli, E., Meat Consumption and Colorectal Cancer: A Review of Epidemiologic Evidence. *Nutr. Rev.* 2001, *59*, 37 – 47.

[7] Skog, K. I., Johansson, M. A., Jägerstad, M. I., Carcinogenic heterocyclic amines in model systems and cooked foods: a review on formation, occurrence and intake. *Food Chem. Toxicol.* 1998, *36*, 879 – 896.

[8] Persson, E., Sjöholm, I., Skog, K., Effect of high water-holding capacity on the formation of heterocyclic amines in fried beefburgers. *J. Agric. Food Chem.* 2003, *51*, 4472 – 4477.

[9] Gu, Y. S., Kim, I. S., Park, J. H., Lee, S. H., Park, D. C., Yeum, D. M., Ji, C. I., Kim, S. H., Wakabayashi, K., Kim, S. B., Effects of seasoning and heating device on mutagenicity and heterocyclic amines in cooked beef. *Biosci. Biotechnol. Biochem.* 2001, *65*, 2284 – 2287.

[10] Murkovic, M., Steinberger, D., Pfannhauser, W., Antioxidant spices reduce the formation of heterocyclic amines in fried meat. *Z. Lebensm.-Unters.-Forsch. A* 1998, *207*, 477 – 480.

[11] Skog, K., Jägerstad, M., Effect of monosaccharides and disaccharides on the formation of food mutagens in model systems. *Mutat. Res.* 1990, *25*, 263 – 272.

[12] Wang, Y., Vuolo, L., Spingarn, N., Weisburger, J., Formation of mutagens in cooked foods. V. The mutagen reducing effect of soy protein concentrates and antioxidants during frying of beef. *Cancer Lett.* 1982, *16*, 179 – 186.

[13] Schoch, A., Gibis, M., Fischer, A., Reduction of mutagenic/carcinogenic heterocyclic amine representative MeIQx by the use of cysteine/ribose during making of hamburger meat patties. *Congress Proceedings, 47th International Congress of Meat Science and Technology*, Krakow, Poland 2001, Vol. II, pp. 248 – 249.

[14] Gibis, M., Schoch, A., Fischer, A., Effect of marinating pretreatment with spices on the formation of mutagenic/carcinogenic heterocyclic amines in fried beef patties. *Congress Proceedings, 47th International Congress of Meat Science and Technology*, Krakow, Poland 2001, Vol. II, pp. 250 – 251.

[15] Salmon, C. P., Knize, M. G., Felton, J. S., Effects of marinating on heterocyclic amine carcinogen formation in grilled chicken. *Food Chem. Toxicol.* 1993, *35*, 433 – 441.

[16] Knize, M. G., Sinha, R., Brown, E. D., Salmon, C. P., Levander, O. A., Felton, J. S., Rothman, N., Heterocyclic Amine Content in Restaurant-Cooked Hamburgers, Steaks, Ribs and Chicken. *J. Agric. Food Chem.* 1998, *46*, 4648 – 4651.

[17] Keating, G. A., Sinha, R., Layton, D., Salmon C. P., Knize, M. G., Bogen, K. T., Lynch, C. F., Alavanja, M., Comparison of heterocyclic amine levels in home-cooked meats with exposure indicators (United States). *Cancer Causes Control* 2000, *11*, 731 – 739.

[18] Tikkanen, L. M., Sauri, T. M., Latva-Kala, K. J., Screening of heat-processed Finnish foods for the mutagens 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline, 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine. *Food Chem. Toxicol.* 1993, *31*, 717 – 721.

[19] Johansson, M., Jägerstad, M., Occurrence of mutagenic/carcinogenioc heterocyclic amines in meat and fish products, including pan residues, prepared under domestic conditions. *Carcinogenesis* 1994, *15*, 1511 – 1518.

[20] Knize, M. G., Dolbeare, F. A., Cunningham, P. L., Moore, D. H., Felton, J. S., Mutagenic activity and heterocyclic amine content of the human diet. In: Adamson, R. H., Gustafsson, J.-Å., Ito, N., Nagao, M., Sugimura, T., Wakabayashi, K., Yamazone, Y. (Eds.), *Heterocyclic Amines in Cooked Foods: Possible Human Carcinogens*, Princeton Scientific Publishing, Princeton, NJ 1995, pp. 30 – 36.

[21] Knize, M. G., Sinha, R., Rothman, N., Brown, E. D., Salmon, C. P., Levander, O. A., Cunningham, P. L., Felton, J. S., Heterocyclic amine content in fast food meat products. *Food Chem. Toxicol.* 1995, *33*, 545 – 552.

[22] Holder, C. L., Cooper, W. M., Churchwell, M. I., Doerge, D. R., Thompson, S., Multi-residue determination and confirmation of ten heterocyclic amines in cooked meats. *J. Muscle Foods* 1996, *7*, 81 – 290.

[23] Gross, G. A., Grüter, A., Quantification of mutagenic/carcinogenic heterocyclic aromatic amines in food products. *J. Chromatogr.* 1992, *592*, 271 – 278.

[24] Borgen, E., Solyakov, A., Skog, K., Effects of precursor composition and water on the formation of heterocyclic amines in meat model systems. *Food Chem.* 2001, *74*, 11 – 19.

[25] Skog, K., Augustsson, K., Steineck. G., Stenberg, M., Jägerstad, M., Polar and non-polar heterocyclic amines in cooked fish and meat products and their corresponding pan residues. *Food Chem. Toxicol.* 1997, *35*, 555 – 565.

[26] Bång, J., Nukaya, H., Skog, K., Blue Chitin Columns for the Extraction of Heterocyclic Amines from Cooked Foods. *J. Chromatogr. A* 2002, *977*, 97 – 105.

[27] Santos, F. J., Barceló-Barrachina, E., Toribio, F., Puignou, L., Galceran, M. T., Persson, E., Skog, K., Messner, C., Murkovic, M., Nabinger, U., Ristic, A., Determination of heterocyclic amines in food products: inter-laboratory studies. *J. Chromatogr. B* 2004, *802*, 69 – 78.

[28] Augustsson, K., Skog, K., Jägerstad, M., Steineck, G., Assessment of the human exposure to heterocyclic amines. *Carcinogenesis* 1997, *10*, 1931 – 1935.

[29] Solyakov, A., Skog, K., Jägerstad, M., Heterocyclic amines in process flavours, process flavour ingredients, bouillon concentrates and a pan residue. *Food Chem. Toxicol.* 1999, *37*, 1 – 11.

[30] Toribo, F., Moyano, E., Puignou, L., Galceran, M. T., Composition of different commercial solid-phase extraction cartridges used to extract heterocyclic amines from lyophilised meat extract. *J. Chromatogr. A* 1999, *880*, 101 – 112.

[31] Knize, M. G., Sinha, R., Salmon, C. P., Mehta, S. S., Dewhirst, K. P., Felton, J. S., Formation of heterocyclic amine mutagens/carcinogens during home and commercial cooking of muscle foods. *J. Muscle Foods* 1996, *7*, 271 – 279.

[32] Skog, K., Knize, M., Felton, J., Jägerstad, M., Formation of new heterocyclic mutagens by heating creatine, alanine, threonine and glucose. *Mutat. Res.* 1992, *268*, 191 – 197.

[33] Tran, N. L., Salmon, C. P., Knize, M. G., Colvin, M. E., Experimental and simulation studies of heat flow and heterocyclic amine mutagen/carcinogen formation in pan-fried meat patties. *Food Chem. Toxicol.* 2002, *40*, 673 – 684.

