**UDK 641.521:577**
Scinetific paper

# FORMATION OF CARCINOGENIC SUBSTANCES DURING HEATING OF FOODS

M. Murkovic[1]

[1]*Graz University of Technology, Institute of Biochemistry, Austria*

***Abstract***

*Heating of foods induces chemical reaction pathways that are not only leading to desired compounds (e.g. aroma and taste active) but also to degradation products that can pose a cancer risk. Especially the Maillard reaction is known for the formation of carcinogenic compounds in some instances. Recently, it was described that the uptake of oxidised lipids can also lead to cancer. However, the active principle is not yet identified and it has been suggested that the aldehydes, peroxides, or epoxides are the chemical structures that induce the changes of the DNA.*

*From the Maillard reaction a number of different potentially toxic substances are formed which comprises the heterocyclic amines, acrylamide, and the furan derivatives. During the last few years a biochemical mechanism was described which activates the furan derivatives (e.g. furfuryl alcohol, HMF) which are then able to form DNA-adducts. Heterocyclic amines are formed from a reaction of amino acids with carbohydrates and creatinine. In contrast to this reaction acrylamide is formed from asparagine in the presence of sugars.*

*The formation of HMF is not so much dependent on high temperatures as the heterocyclic amines or acrylamide. It is also formed during storage of carbohydrate rich foods. Other furan derivatives like furfuryl alcohol need higher temperatures as well.The concentration of these compounds covers a wide range from low ng/g in the case of heterocyclic amines to µg/g in the case of acrylamide and mg/g in the case of HMF and other furans. This means that even if the carcinogenic potential of the furans is low the high concentration and ubiquitous occurrence results in a chronic and high exposure which can also contribute significantly to the cancer risk of heated foods.*

***Key words:*** *heterocylic amines, acrylamide, furan derivatives, carcinogens, formation*

## 1. INTRODUCTION

In some cases, heating of foods results in significant amounts of carcinogenic substances. Depending on the precursors and the chemical as well as physical environment chemical reactions will take place. High temperatures are needed to obtain a good sanitizing of the food and optimize the changes in texture and aroma. Some of the chemicals formed can be harmful to humans i.e. they can contribute to carcinogenesis. When meat is heated the reactions of amino acids with creatinine –

in presence or absence of carbohydrates – lead to the formation of heterocyclic aromatic amines. Of the 20 described different heterocyclic amines around 10 are known to be carcinogenic. Normally these substances are activated with phase I and phase II enzymes leading to highly reactive metabolites that can form adducts with the DNA. From epidemiology and animal tests it can be concluded that these heterocyclic amines contribute to the increased (e.g. colon) cancer risk.

This type of activation (involving cytochrome P450 enzymes as well as N-acetyl transferases and sulfotransferases) seems to be a common principle for different chemical structures. Acrylamide is activated to glycidamide and furfuryl alcohol structures are sulfatated. In all of the cases highly reactive structures are the results of these enzymatic pathways.

The results of the recent scientific work show that it will be difficult to develop strategies to mitigate the formation of these substances as they are parallel of reactions in the pathways leading to the aromatic principle of the foods.

## 2. HETEROCYCLIC AROMATIC AMINES

Formation of HAs in foods

Pearson et al. proposed two different pathways for free radical formation [1]. One involves bimolecular ring formation from the enaminol form of the glycoaldehyde alkylimine that is followed by oxidative formation of the free radical. The other pathway involves formation of N,N1-diaklylpyrazinium ions from glyoxal monoalkylimine followed by reduction to produce the free radicals. The respective intermediates (glycoaldehyde alkylimine and glyoxal monoalkylamine) are formed by reacting glycoaldehyde and glyoxal with amino compounds. The reactions help to explain the formation of imidazoquinoxaline meat mutagens and their predominance in fried fish and why these mutagens are present in larger quantities in fried ground beef than the imidazoquinoline-type meat mutagens. (Fig. 1)

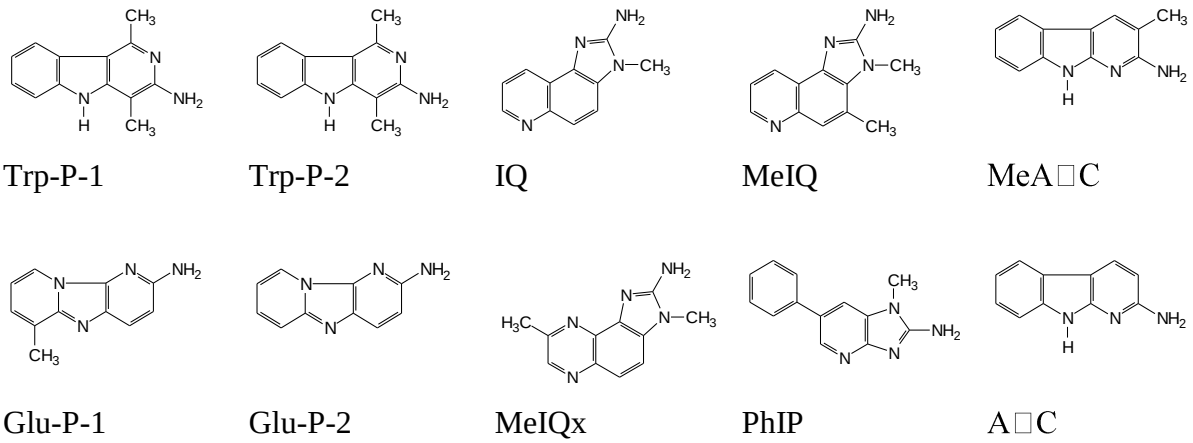


*Fig. 1. Structures of HAs being carcinogenic to rodents.*

For evaluation of the mechanism of formation model systems were developed which allowed identifying the precursors necessary for HA formation [2]. The first published model systems in which mutagenic compounds were identified was a pyrolysis reaction of amino acids and proteins. Other food constituents did not form mutagenic substances during pyrolysis [3]. The substances identified were the same as found in the charred parts of roasted or grilled meat and fish [4]. Nucleic acids, starch, or oil did not form mutagenic substances during pyrolysis. Heating of single amino acids also resulted in the formation of mutagenic substances that were identified as heterocyclic aromatic amines. In general, the products of pyrolysis are assigned as non-polar HAs. In contrast, harman and norharman are both formed at lower temperatures and Trp-P-1 was also shown to occur in cooked meat, which was prepared at lower temperatures [5]. The polar HAs (IQ, MeIQ, MeIQx, PhIP) are formed at normal cooking temperatures. These mutagens were identified in fried meat and fish. They were also found in meat products e.g. in meat extract that is extracted at comparably low temperatures but using longer times for processing [6].

Most polar HAs are formed from creatine/creatinine, amino acids and carbohydrates. The heating of the reaction mixture was done as a dry powder or dissolved in diethylene glycol. Both methods of heating lead to the same heterocyclic aromatic amines with more or less the same quantitative results. All this work is reviewed in [7].

When using meat juice for model systems the complexity increases since several polar and non-polar HAs are formed [8 – 10]. Since the composition of the precursors (amino acids, glucose, creatine) simulates the chemical environment in the meat much better than a solution of single amino acids in diethylene glycol the results are more relevant but much more complicated to interpret. Depending on the type of meat from which the juice is derived the amino acid composition and the glucose and creatine content vary to a great extend. This results in a specific pattern of the formed HAs.

## Occurrence of HAs in foods

Although the HAs are found ubiquitous it is only the uptake of heated meat and fish products that contributes significantly to the exposure since the concentrations in other foods or in the environment are extremely low. The analysis in meat and fish showed concentrations in the range of 0 to 10 ng/g. The amount of HAs formed depends on the cooking method and type of meat. In tables 1 and 2 the values are summarised. It can be seen that the IQ and IQx type substances are formed in the low ppb range. In meat extracts and gravies the concentrations can be much higher. This is a result of the higher concentration of the precursors from the meat juice, the higher temperature and a concentration effect of the evaporating water. PhIP that is formed by a different mechanism occurs in substantial higher amounts. The non-polar HAs - which are formed as pyrolysis products of amino acids can also be very high. Norharman and harman concentrations are comparably high but do not contribute to carcinogenesis. Some experiments showed that especially very intensively fried meat can contain much higher amounts of polar and non-polar HAs [11].

Totsuka and co-workers [12] found remarkable high concentrations of harman and norharman in flame broiled meat (chicken norharman 622 ng/g, harman 133 ng/g; beef: norharman 795 ng/g, harman 169 ng/g; mutton: norharman 458 ng/g, harman 68 ng/g).

*Table 1. Content of polar HAs in cooked foods (ng/g) [11]*

| | MeIQx | IQ | 4,8-DiMeIQx | PhIP |
|---|---|---|---|---|
| Red meat | 0 - 10 | 0 - 2 | 0 - 5 | 0 - 35 |
| Poultry | 0 - 3 | 0 - 1 | 0 - 3 | 0 - 10 |
| Fish | 0 - 2 | 0 - 1 | 0 - 1 | 0 - 10 |
| Meat extract, gravy | 0 - 80 | 0 - 15 | 0 - 9 | 0 - 10 |

*Table 3. Content of non-polar HAs in cooked foods (ng/g) [11, 13]*

| | A□C | Trp-P-1 | Trp-P-2 | Norharman | Harman |
|---|---|---|---|---|---|
| Red meat | 0 - 20 | 0 - 1 | 0 - 2 | 0 - 30 | 0 - 20 |
| Poultry | 0 - 1 | 0 - 2 | 0 - 1 | 0 - 13 | 0 - 12 |
| Fish | 0 - 10 | 0 - 1 | n.d. | 0 - 200 | 0 - 130 |
| Meat extract, gravy | 0 - 3 | 0 - 5 | n.d. | 0 - 100 | 0 - 120 |

Influence of cooking on the content of HAs

All normally eaten meat products, which are derived from beef, pork or poultry, have to some degree mutagenic properties. The way of preparation influences the content of the HAs. The parameters with the highest influence are the cooking temperature and cooking time. It was shown several times that with increasing temperature the content of HAs increases also. In some of these experiments it was found that the mutagenic activity reaches a plateau at 200 - 250 °C. For example the increase of the pan temperature of 50 °C during frying of meat leads to a doubling of the HA content between 200 and 300 °C. Although the content of the HAs can be reduced substantially with lower frying temperatures there is definitely no temperature limit where no HAs are formed. The influence of temperature on the formation of HAs in poultry meat was also shown. Having a low frying temperature of about 150 °C only low amounts of IQ, MeIQ, MeIQx, 4,8-DiMeIQx and PhIP are formed [14].

The HAs are not only found in the crust of the heated meat but also in the gravy or pan residue. When frying hamburgers about 23 % of the mutagenic activity is found in the gravy [39]. If

pork is sliced and fried for 10 min at 200 to 250 °C half of the mutagenic activity is found in the crust and gravy respectively [14]. The analysis of 14 cooked meat dishes and the pan residues showed that up to a temperature of 150 °C the total content of HAs was below 1 ng/g. A temperature up to 175 °C led to a content of about 2 ng/g. The highest amounts of HAs were found in those foods that were heated at 200 to 225 °C [16]. Other experiments showed that the mutagenic activity is predominantly formed in the pan residue when minced pork is heated to 200 °C for 5 - 25 min [17]. If minced beef and poultry is heated to 220 °C for 10 min 20 - 40 % of the mutagenic activity is found in the pan residue [18].

Besides temperature and heating time the composition of the meat plays a crucial role in the formation of HAs. Especially the availability of the precursors (amino acids, creatinine, carbohydrates) influences the formation of the HAs. Experiments to study the effect of precursors were mainly done in model systems. The influence of the type of fat [19] as well as the oxidative status of the frying fat shows an influence on the formation. In several publications it was stated that the presence antioxidants reduces the content of HAs in meat.

The influence of the cooking method on the HA content is shown in table 3. In this experiment bacon was fried in a pan. In the pan the content of HAs was much higher compared to grilled bacon. This is due to the fact that the HAs are enriched in the fat phase, which drips off during grilling.

Investigations on heat and mass transfer in chicken breasts revealed that besides a high cooking temperature the rate of drip loss has a significant influence on the formation of PhIP [20]. Further work showed that a reduction of cooking loss – which mainly refers to water – leads to a reduced formation of PhIP, MeIQx and 4,8-DiMeIQx. In their experiments Persson and co-workers used sodium chloride and sodium tripolyphosphate to increase the water holding capacity of the meat during cooking and thus the reduction of cooking loss [21]. A further addition of polymeric carbohydrates to beef burgers even more reduced the PhIP formation [22]. Dietary fibres are not only interesting for their potential to reduce HA formation during cooking but also for their influence on HA metabolism in the digestive tract. It was shown that the uptake in the intestinal tract was reduced which was attributed to a decreased transit time of the HAs. Additionally, the concentration of the HAs in the colon is reduced when a diet rich in fibre is eaten. [23, 24]

## 3. ACRYLAMIDE

The main precursor for the formation of acrylamide in foods is asparagines. In presence of sugars acrylamide is formed via the Maillard reaction with an oxazolidinone as key intermediate [39]. This reaction occurs at temperatures that are typical for frying and roasting. In coffee asparagine is one of the dominating amino acids. Together with sucrose that is cleaved to glucose and fructose at roasting conditions the reaction pathway for the formation of acrylamide is initiated. The roasting of coffee is done at high temperatures in the range of 240-300 °C [25]. For laboratory roasting, 24 min at 220-230 °C was evaluated as optimal conditions for the acceptable sensory properties for the coffee beverage [26]. During roasting of the green coffee the typical dark brown

to almost black colour develops. During the Maillard reaction, Strecker degradation, and pyrolysis reactions of carbohydrates and amino acids, a great variety of monomeric and polymeric compounds are formed that influence the aroma, taste, and other sensory properties of the beverage.

Acrylamide was mentioned by numerous researchers to be one of the hazardous compounds formed during the roasting, baking, and frying of foods. It is carcinogenic to laboratory rodents and is described by the International Agency for Research of Cancer as a probable carcinogen to humans [27]. In the human body acrylamide is oxidized to the epoxide glycidamide (2,3-epoxypropionamide) via an enzymatic reaction involving cytochrome P450 2E1 [28]. Glycidamide has been shown to form adducts with amino groups of the DNA. It was shown that high levels of acrylamide can cause mutations and cellular transformation [29].

Coffee as a research object is important because of its high consumption in some countries and therefore possible hazardous influence on human health. The contribution of coffee to the dietary daily intake of acrylamide is high in countries with a high coffee consumption such as Norway and Sweden, where it can reach 30 % [30], Denmark, where it can be 20 % [31], and Switzerland, where it can reach 36 % [32]. Although coffee beans are roasted at quite high temperatures, the amounts of acrylamide found in the roasted beans and ground coffee are reported to be low [33]. There are no significant differences in acrylamide formation in normal or decaffeinated coffee.

In coffee, acrylamide is formed in high concentrations during the first minutes of roasting, resulting in > 7000 ng/g. The increase of roasting time leads to the degradation of acrylamide. Kinetic models and spiking experiments with isotope-labelled [$^{14}$C]acrylamide showed that >95 % of the acrylamide is lost during roasting [34, 35]. Similar results were reported by Lantz and co-workers [36]. However, the roasting conditions have an important influence on the typical coffee aroma and taste that are desirable to consumers. Therefore, the optimization of the roasting conditions with respect to a reduction of the acrylamide formation and maintaining the product quality has not been realized yet [37]. As it was reported in ref 16, during the brewing step of the coffee beverage, almost all acrylamide present in the coffee powder is transferred to the liquid phase of the coffee drink, due to its high solubility in water. In analyzed grounds after brewing no acrylamide was detected. It seems that all acrylamide available in coffee powder is transferred to the water, where it is quite stable. No significant decrease in acrylamide levels was observed after several hours of heating. In contrast to filtered coffee, the acrylamide is not transferred completely to the beverage when espresso coffee is prepared. It has been noted that during storage the acrylamide content decreases [28, 36, 38]. In instant coffee acrylamide content is reduced by 67 % within 1 year of storage and in roasted coffee by 28 % within 7 months [28]. It was reported [36] that the storage temperature has a significant influence on the acrylamide Degradation in vacuum-packed roasted and ground coffee.

## 4. FURANS

5-Hydroxymethyl-2-furoic acid (HMFA) is the main metabolite of 5-hydroxymethyl-2-furfural (HMF) in the body and eliminated renally. HMF is formed by an acid catalysed degradation of

reducing sugars or via the Maillard reaction. This reaction occurs when reducing sugars react with amino acids or proteins [50]. The progress of the Maillard reaction is strongly influenced by the reaction time, the temperature, the concentration of the precursors and the pH. The Maillard reaction takes place in foods during heating and storage and has an important influence on the appearance and taste of food. Additionally to foods the Maillard reaction also takes place in the human body and influences a wide variety of physiological functions [47, 57].

The content of HMF in food, like dried fruits, bread, jam, coffee, caramel, juice and others was analysed by several groups. Bachmann and co-workers [41] estimated 3.2–220 mg/kg HMF in bread whilst Ramìrez-Jiménez and his co-workers [54] analysed Spanish bread and found HMF concentrations in the range of 2.2–68.8 mg/kg. In dried fruits HMF was found from 1 to 2200 mg/kg by Murkovic and Pichler [52]. The highest concentration was found in dried plums up and in jam made from plums with contents up to 2200 mg/kg and 1200 mg/kg, respectively. In coffee the concentration of HMF is in the range of 300–1900 mg/kg [51, 52]. Bachmann and co-workers [41] also analysed meat and meat products and found very low concentrations below 0.9 mg/kg product whilst high HMF amounts were found in caramel with 110–9500 mg/kg and 26–3500 mg/kg in dried fruits. In ready to drink coffee they estimated 3.2–72 mg/L. Ameur and co-workers [40] found HMF concentrations in commercially produced cookies from 0.5 to 74.6 mg/kg. In infant cereals HMF was found in the range of 0.4–65.5 mg/kg [42, 55]. Much higher content of HMF was detected in breakfast cereals in the range of 3.7–193.3 mg/kg [43].

The human intake of HMF has been reported in several publications. Ulbricht and co-workers [56] indicated a daily intake of HMF with 150 mg/person (2.5 mg/kg body weight). Janzowski et al. [45] published a daily intake in the range from 30 to 60 mg (0.5–1 mg/kg body weight). The group of Zhang [58] demonstrated that HMF induced aberrant crypt foci (precursor of colon cancer). Janzowski et al. [45] have found a weak genotoxic and mutagenic capability of HMF in *in vitro* studies.

Not only Glatt and Sommer [44] suggested the biotransformation of HMF but also Prior, Wu, and Gu [53] have detected HMFA in rat urine with 36.9 % of the ingested HMF. Jellum et al. [46] determined that fructose solutions used for parenteral nutrition contain up to 1.2 g/L HMF. HMF is formed from fructose at a pH below 4 during sterilisation at 120 C. Patients who obtained these fructose solutions metabolized 50 % of the HMF to HMFA and 2,5-furan dicarboxylic acid. The analytical methods used in earlier studies comprised GC–MS of derivatized HMFA (methylated of trimethyl-silylated) or LC with coulometric detection or MS/MS. Liebich and Forst [48] analysed the urine of eight healthy individuals using GC–MS after methylation with diazomethane. Quantitative data were not given in this publication. Mardens et al. [49] used GC–MS after purification of the samples on an ion exchange SPE and TMCS/BSTFA derivatization.

## 5. CONCLUSIONS

The type of substances described here are all formed in parallel to the substances that contribute to the typical aroma of heated foods. Therefore, these substances cannot be avoided during

traditional food preparation. In some cases, the exposure can be quite high if the food is overcooked or if certain types of foods (in case of HMF) having high concentrations of sugars are stored for a long time at elevated temperatures. To reduce the exposure, it is suggested to change the traditional cooking to lower temperature and shorter cooking and reduce the duration and temperature of storage. If some typical foods containing high amounts of the substances discussed they should be avoided.

## REFERENCES


[1] Puignou, L., Casal, J., Santos, F. J., Galceran, M. T. *J. Chromatogr. A* 1997, 769 293-299.

[2] Casal, J., Mendes, E., Fernandes, J.O., Oliveira, M.B.P.P., Ferreira, M.A. *J. Chromatogr. A* 2004, 1040, 105-114

[3] 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.*J. Chromatogr. B* 2004, 802, 69-78

[4] Pearson, M., Chen, C., Gray, J. I., Aust, S. D. *Free Rad. Biol. Med.* 1992, 13, 161-167

[5] Murkovic, M.*J. Chromatogr. B*2004, 802, 3-10

[6] Nagao, M., Honda, M., Seino, Y., Yahagi, T., Kawachi, M. *Cancer Lett.* 1977, 2, 335-339

[7] Nagao, M., Honda, M., Seino, Y., Yahagi, T., Sugimura, T. *Cancer Lett.* 1977, 2, 221

[8] Arvidsson, P., vanBoekel, M.A.J.S., Skog, K., Solykaov, A., Jägerstad, M. *J. Food Sci.* 1999, 64, 216

[9] Bordas, M., Moyano, E., Puignou, L., Galceran, M. T. *J. Chromatogr. B* 2004, 802, 11-17

[10] Jägerstad, M., Skog, K., Grivas, S., Olsson, R. *Mutat. Res.* 1991, 259, 219

[11] Arvidsson, P., vanBoekel, M.A.J.S., Skog, K., Jägerstad, M. *J. Food Sci.* 1999, 64, 216-225

[12] Borgen, E., Solyakov, A., Skog, K. *Food Chem.* 2001, 74, 11-17

[13] Messner, C., Murkovic, M. *J. Chromatogr. B*2004, 802, 19-26

[14] Miller, J., Buchanan, R. L. *Food Chem. Toxicol.* 1988, 21, 319-323

[15] Nielsen, P., Vahl, M., Gry, J. *Z. Lebensm. Unters. Forsch. A* 1984, 187, 451-456

[16] Overvik, E., Nilsson, L., Fredholm, L., Levin, O., Nord, C. E., Gustafsson, J. A. *Mutat. Res.* 1984, 135, 149-157

[17] Murkovic, M., Friedrich, M., Pfannhauser, W. *Z. Lebensm. Unters. Forsch. A* 1997, 205, 347-350

[18] Felton, J., Healy, S., Stuermer, D., Berry, C., Timourian, H., Morris, M., Bjeldanes, L. *Mutat. Res.* 1981, 88, 33-44

[19] Skog, K., Augustsson, K., Steineck, G., Stenberg, M., Jägerstad, M. *Food Chem. Toxicol.* 1997, 35, 555-565

[20] Berg, Overvik, E., Gustafsson, J. *Food Chem. Toxicol.* 1990, 28, 421-426

[21] Knize, M. G., Shen, N., Felton, J. *Mutagenesis* 1988, 3, 503-508

[22] Johansson, M., Fredholm, L., Bjerne, L., Jägerstad, M. *Food Chem. Toxicol.* 1995, 33, 993-1004

[23] Persson, E., Sjöholm, I., Skog, K. *Eur. Food Res. Technol.* 2002, 214, 455-459
[24] Persson, E., Sjöholm, I., Skog, K. *J. Agric. Food Chem.* 2003, 51, 4472-4477
[25] Cirilo, M. P. G., Coelho, S., Araújo, C. M., Goncalves, F. R. B., Nogueira, F. D., Gloria, M. B. A. *Food Chem.* 2003, 82, 397–402.
[26] Mendes, L. C., de Menezes, H. C., Aparecida, M., da Silva, A. P. *Food Qual. Pref.* 2001, 12, 153–162
[27] International Agency for Cancer Research. Some Naturally Occurring Substances: Food Items and Constituents, Heterocyclic Aromatic Amines and Mycotoxins, IARC: Lyon, France, 1993, 56, 163-242
[28] Friedman, M., Mottram, D. In Chemistry and Safety of Acrylamide in Food, Advances in Experimental Medicine and Biology 561, Springer, New York, 2005, 79
[29] Park, J., Kamendulis, L. M., Friedman, M. A., Klaunig, J. *Toxicol. Sci.* 2002, 65, 177–183
[30] Svensson, K., Abramsson, L., Becker, W., Glynn, A., Hellenäs, K.-E., Lind, Y., Rosén, J. *Food Chem. Toxicol.* 2003, 41, 1581–1586
[31] Granby, K., Fagt, S. *Anal. Chim. Acta* 2004, 520, 177–182
[32] Dybing, E. et al., *Food Chem. Technol.*2005,43, 365–410
[33] Friedman, M. *J. Agric. Food Chem.* 2003, 51, 4504–4526
[34] Skog, K., Alexander, J. In Acrylamide and Other Hazardous Compounds in Heat-Treated Foods, Woodhead Publishing, Cambridge, U.K., 2006, 36
[35] Taeymans, D. et al., *Crit. Rev. Food Sci. Nutr.* 2004, 44, 323–347
[36] Lantz, I., Ternité, R., Wilkens, J., Hoenicke, K., Guenther, H., van der Stegen, G. H. D. *Mol. Nutr. Food Res.* 2006, 50, 1039–1046
[37] Guenther, H., Anklam, E., Wenzl, T., Stadler, R. *Food Addit. Contam.* 2007, 24, 60–70
[38] Andrzejewski, D., Roach, J. A. G., Gay, M. L., Musser, S. M. *J. Agric. Food Chem.* 2004, 52, 1996–2002
[39] Yaylayan, V. A., Wnorowski, A., Perez Locas, C. *J. Agric. Food Chem.* 2003, 51, 1753–1757
[40] Ameur, A. L., Trystram, G., Birlouez-Aragon, I. *Food Chemistry*, 2006, 98, 790–796
[41] Bachmann, S., Meier, M., Känzig, A. *Lebensmittelchemie* 1997, 51, 49–50
[42] Fernandez-Artigas, P., Guerra-Hernández, E., García-Villanova, B. *J. Agric. Food*
[43] *Chem.* 1999, 47, 2872–2878.
[44] García-Villanova, B., Guerra-Hernández, E., Martinez Gomez, E., Montilla, J. *J. Agric. Food Chem.* 1999, 41, 1254–1255
[45] Glatt, H., Sommer, Y. In K. Skog J. Alexander (Eds.), Acrylamide and other health
[46] hazardous compounds in heat-treated foods, Woodhead, Cambridge, 2006, 328–357
[47] Janzowski, C., Glaab, V., Samimi, E., Schlatter, J., Eisenbrand, G. *Food Chem. Toxicol.*, 2000, 38, 801–809
[48] Jellum, E., Borresen, H. C., Eldjarn, L. *Clinica Chimica Acta* 1973, 47, 191–201
[49] Ledl, F., Schleicher, E. *Angewandte Chemie* 1990, 102, 597–626
[50] Liebich, H. M., Forst, C. J. Chromatogr. 1990, 525, 1–14

[51] Mardens, Y., Kumps, A., Planchon, C., Wurth, C. *J. Chromatogr*.1992, 577, 341–346
[52] Mauron, J. *Progress in Food and Nutrition Sciences* 1981, 5, 5–35
[53] Murkovic, M., Bornik, A. M. *Molecular Nutrition and Food Research* 2007, 51, 390–394
[54] Murkovic, M., Pichler, N. *Molecular Nutrition and Food Research* 2006, 50,
[55] 842–846
[56] Prior, R. L., Wu, X., Gu, L. *J. Agric. Food Chem.* 2006, 54, 3744–3749
[57] Ramírez-Jiménez, A., Guerra-Hernández, E., Gracía-Villanova, B. *J. Agric. Food Chem.* 2000, 48, 4176–4181
[58] Ramírez-Jiménez, A., Guerra-Hernández, E., Gracía-Villanova, B. *Food Chemistry* 2003, 83, 219–225
[59] Ulbricht, R. J., Northup, S. J., Thomas, J. A. *Fundamental and Applied*
[60] *Toxicology* 1984, 4, 843–853
[61] Weigel, K. U. Dissertation, TU Dreseden, Germany, 2004
[62] Zhang, X. M., Chan, C. C., Stamp, D., Minkin, S., Archer, M. C., Bruce, W. R.
[63] *Carcinogenesis* 1993, 14, 773–775