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Review paper
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Formation of carcinogenic substances during heating of foods

The formation of some selected carcinogens during cooking is associated with some certain types of foods. The heterocyclic amines are mainly found in meat and fish that are cooked at temperatures above 150°. Acrylamide is formed in foods containing asparagine and reducing sugars which are present in potatoes, cereals and similar products. The formation of acrylamide needs high temperatures similar to the HCAs which are occurring during baking, frying, and roasting. In contrast to the other two types of substances 5-hydroxymethyl-2-furfural is formed at lower temperatures and even during storage from carbohydrates in presence of amino acids or from fructose by direct dehydration. Since all these compounds are by-products of the aroma forming reactions it is difficult to find ways to reduce the content in foods without changing the characteristics of the food products. A proper selection of the raw materials and well controlled cooking/processing procedures in combination with a reduced uptake of some highly contaminated foods can give us a possibility to reduce the exposure.

Key words: *carcinogenic substances, contaminated foods, heating*

INTRODUCTION

Since the 1970s carcinogenic compounds that are formed endogenously during heating of foods were identified and characterized in detail. In addition to the well known benzo[a]pyrene the heterocyclic aromatic amines from amino acid pyrolysates were identified (Trp-P-1, Trp-P-2, Glu-P-1, Glu-P-2, AαC, MeAαC) (Sugimura et al., 1977; Yamamoto et al., 1978; Yoshida et al., 1978). In a next step the compounds IQ and MeIQ were detected in broiled sardines (Kasai et al., 1980) and MeIQx in fried beef (Kasai et al., 1981). The precursors that are a prerequisite for the formation of quinolines and quinoxalines were identified by Jägerstad and coworkers (1984) being carbohydrates, amino acids, and creatinine. During the following years twenty of these compounds were identified and ten of them were shown to be carcinogenic. The formation of these compounds is linked to the presence of the precursors (carbohydrates, amino acids, creatine) and cooking temperatures of above 150 °C which are used at grilling, frying, broiling etc.

In 2002 the publication of the findings of a Swedish group showed that acrylamide is present in high amounts in foods. Especially heated products from potatoes and cereals contain rather high concentrations. The precursors for this product were identified as asparagine and sugars (Mottram et al., 2002). As it is well established that acrylamide is a toxic and carcinogenic compound a huge research effort was undertaken to evaluate the exposure and from these data the toxicological risk of acrylamide was derived. Based on the modes of action, Tardiff and co-workers (2010) a non-linear dose-response approach was applied for neurotoxicity and carcinogenicity. They concluded that the tolerable intakes of acrylamide should be set at 2.6 µg/kg BW/d and 16 µg/kg BW/d based on acrylamide and glycidamide, respectively, to avoid a cancer risk. This would be equivalent to 182 µg for a 70 kg human as a tolerable daily intake (TDI) for carcinogenic levels. The TDI for neurotoxicity from acrylamide was estimated to be 40 µg/kg BW/d which would be equivalent to 2,800 µg/d for a 70 kg human. The margins of exposure (MoE) were calculated for average acrylamide consumers to be 300 and 500 based on acrylamide and glycidamide, respectively; for cancer, the MoE for average acrylamide consumers was estimated to be 200 and 1200 based on acrylamide and glycidamide, respectively.

5-Hydroxymethyl-2-furfural (HMF) is a compound that is formed via different mechanisms from either glucose in presence of amino substituted compounds or from fructose directly via elimination of water. The concentrations can reach very high levels up to g/kg. The toxicity tests of HMF showed that this compound is safe but recent experiments with sulfotransferases showed that HMF can be activated and become mutagenic (Glatt, 1997; Durling et al., 2009).

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Heterocyclic amines

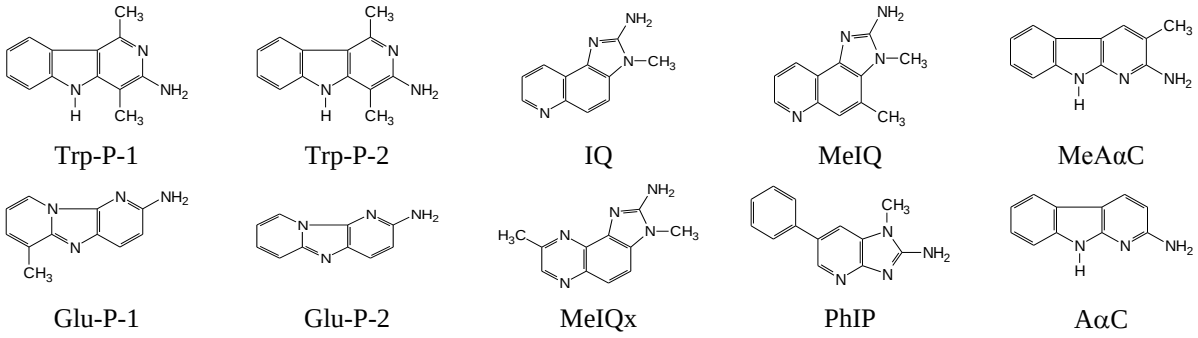

Figure 1 - Structures of carcinogenic HAs

ANALYSIS OF HETEROCYCLIC AROMATIC AMINES

The analysis of these substances is a twofold challenge for the food chemist. The first challenge is the very low concentration in the heated meat and the second is the highly complex matrix that needs several clean-up steps before the analysis by HPLC can be carried out. During the last years the method developed by Gross (Gross and Grüter, 1992) became internationally accepted.

Extraction and clean up of HCAs: The first extraction with dichloromethane or ethyl acetate is done after saponification. Subsequently a two step solid phase extraction (SPE) is carried out which includes a strong cation exchanger (e.g. PRS) and subsequently a C18-material. A very interesting SPE material known as blue cotton can also be used. Blue cotton is a trisulfo copper phthalocyanin complex linked to cellulose. This planar system has a strong and selective interaction with the HAs (Hayatsu et al., 1983). Skog reviewed the published literature on the use of blue cotton for cleanup of HA extracts from heated meat and fish (Skog, 2004). Recently a method using solid-phase microextraction coupled with HPLC and UV detection was published (Cardenes et al., 2004). The authors could show that this technique being a well established clean up method in gas chromatography could also be used in HPLC analysis of polar compounds.

Chromatography and detection of HCAs: The chromatographic separation and detection is a crucial point in the analysis. In the recent years mass spectrometry especially MS-MS was shown to be the method of choice since the selectivity and sensitivity is excellent for complex matrices (e.g. Busquets et al., 2007). Another alternative that was published recently is the use of TLC combined with UV and fluorescence detection (Jautz et al., 2008).

OCCURRENCE OF HCAS IN FOODS

Heterocyclic amines (HCA) are formed from carbohydrates and amino acids at high cooking temperatures that are occurring during frying, grilling, and broiling. In the case of the polar HCAs creatinine is an additional precursor which limits the occurrence to food containing creatine which is mainly meat and fish. The concentration range of these compounds is in the low ng/g range but in some cases, especially in meat which is very well done the concentration of IFP can reach 25 ng/g. In the same sample 7-MeI*g*Qx was found at levels of up to 11 ng/g and PhIP can even reach concentrations up to 300 ng/g (Ni et al., 2008).

In addition to earlier work a huge range of foods was investigated for the content of the HCAs. Sun et al. (2010) investigated mutton shashlik – a traditional and popular dish in China – contains typically low amounts of the carcinogenic HCAs being below 2 ng/g (AαC, PhIP). Tai and his group (2001) analyzed HCAs in fried fish fibre. Depending on the recipe (monosodium glutamate, ascorbic acid) the concentrations of AαC and MeAαC were in the range of 1 to 10 ng/g. The addition of higher amounts of ascorbic acid resulted in a complete elimination of the two compounds. Oz (2011) analyzed Turkish meat balls by ultra fast liquid chromatography with UV detection and found IQ, IQx, MeIQ, PhIP, AαC, MeAC in concentrations of up to 3.3 (IQx) in samples from different regions in Turkey. Barbecued sardines and Atlantic salmon were analysed by Costa and coworkers (2009). This group identified Glu-P-1, Trp-P-1, Trp-P-2, MeIQx, PhIP, AαC, and MeAαC in the different samples at concentrations of up to 13 ng/g. Especially the non-polar HCAs like PhIP, AαC, MeAαC, Trp-P-1, and Trp-P-2 were occurring at comparably high concentrations in some samples. Different species of trout were analyzed by the group of Oz (2007). In this publication extremely low concentrations of IQ and 4,8-DiMeIQx were reported being below 0.1 ng/g. In this case e.g. PhIP was not detected.

The efforts to mitigate the concentrations of the HCAs comprised the use of different marinades like fruit extracts (Cheng et al., 2007), green tea (Quelhas et al., 2010), garlic, onion, as well as lemon juice (Gibis, 2007), spice extracts (Damasius et al., 2011), red wine (Busquets et al., 2006; Melo et al., 2008), and beer (Melo et al., 2008).

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 (Nielsen et al., 1984). 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 (Murkovic et al., 1997).

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 (Felton et al., 1981). 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 (Murkovic et al., 1997). 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. Temperatures of 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 (Skog et al., 1997). 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 (Berg et al., 1990). 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 (Knize et al., 1988). Janoszka and co-workers (2009) found similar concentrations in the gravy and in the meat and in some cases even lower concentrations in the gravy. In their manuscript concentrations were reported being up to 10 ng/g with MeIQ showing the highest concentrations.

ACRYLAMIDE

The identification of acrylamide goes back to the year 2002 when a Swedish group published the finding of acrylamide in heated foods, especially in fried potato products like chips and crisps. Mottram and his co-workers published in 2002 that the main precursor is asparagine. The obviously simple mechanism of formation which includes a decarboxylation and ammonia elimination was evaluated in detail by the group of Yaylayan (Perez-Locas and Yaylayan, 2008). In these studies it was emphasised that a 5-oxazolidinone is an important intermediate in the formation reaction. This intermediate is a general intermediate which is similar in reactions of other amino acids.

ANALYSIS OF ACRYLAMIDE

Since acrylamide is a highly polar compound that is easily dissolved in water, this is used as extraction solvent. For purification of the extract normally a multimode solid phase extraction material is used. The purified extract is directly analysed by HPLC-MS/MS. The analysis by GC-MS is also possible either directly or after bromination. To increase the precision the use of a $^{13}C_3$-acrylamide or D_3-acrylamide as internal standard is recommended. For chromatography a Hypercarb(R) column can be used with water as eluent; other possibilities comprise hydrophilic end-capped reversed phase columns. A detailed overview and discussion of the analytical methods currently used is given by Eriksson (2005).

CONTENT OF ACRYLAMIDE IN FOODS AND DIETARY EXPOSURE

In 2011 the European Food Safety Agency (EFSA) published a report on the monitoring of acrylamide in foods during the recent years. Twenty three Member States and Norway submitted a total of 10,366 acrylamide results for the three-year period 2007 to 2009. In 2009, mean acrylamide levels ranged from 37 μg/kg for soft bread to 1504 μg/kg for substitute coffee, while the highest 95th percentile and maximum levels were reported for substitute coffee at 3,976 and potato crisps at 4,804 μg/kg, respectively. Based on the three years of information available it could be identified that acrylamide decreased in crackers, infant biscuits and gingerbread over the three years and increased in crisp bread and instant coffee. From this overview it was concluded that the mean acrylamide exposure in Europe ranges between 0.31 and 1.1 μg/kg BW/d for adults, between 0.43 and

1.4 μg/kg BW/d for adolescents (11-17 years), between 0.70 and 2.05 μg/kg BW/d for children (3-10 years) and between 1.2 and 2.4 μg/kg BW/d for toddlers (1-3 years). The major contributors to exposure for adults were fried potatoes (including French fries), coffee, and soft bread whereas for adolescents and children they were fried potatoes, soft bread and potato crisps or biscuits.

The mean dietary exposure to acrylamide for adults (>18 years) in Europe was estimated to range between 0.31 and 1.1μg/kg BW/d. High exposure at the 95^{th} percentile varied from 0.58 to 2.3 μg/kg BW/d. These results are similar to the range reported in the latest JECFA acrylamide risk assessment report (FAO/WHO, 2010) in which mean and 95^{th} – 97.5^{th} percentile estimates ranged between 0.2 and 1 μg/kg BW/d and 0.6 to 1.8 μg/kg BW/d, respectively, for the general adult population. In this study fried potatoes (including French fries), soft bread and roasted coffee were identified as the major contributors to the overall adult acrylamide exposure. JECFA (FAO/WHO, 2010) identified French fries, potato crisps, bread and biscuits as the main contributors for the general adult population. (EFSA, 2011)

MITIGATION EFFORTS

Since the finding of acrylamide in foods a series of experiments were carried out to mitigate the formation of this potentially harmful substance. In a review Friedman and Levin summarized all published concepts to reduce the content of dietary acrylamide (2008). A mitigation could be achieved by several ways which comprise the proper selection of raw materials having optimized contents of the precursors, removing the precursors before processing, enzymatic hydrolysis of asparagine to aspartic acid using asparaginase, optimizing the process and storage conditions, adding components that are known to reduce the acrylamide content either by inhibiting the formation or enhancing further reactions of acrylamide, removing acrylamide from the foods, and reducing the in vivo toxicity. The CIAA has published the acrylamide toolbox that should help the food producers to change their processes for obtaining acrylamide reduced products. The latest issue can be found at http://www.fooddrinkeurope.eu/uploads/publications_documents/Toolboxfinal260911.pdf.

Especially the use of asparaginase which was investigated in detail by the group of Ciesarova shows very promising applications in potatoes (Ciesarova et al., 2006). Similar applications were published by Hendriksen and co-workers in 2009.

HYDROXYMETHYLFURFURAL

5-Hydroxymethyl-2-furfural is a compound that was investigated for its toxicity – mainly because it was found in solutions for parenteral nutrition. The toxicity level which was established was estimated to an acute oral LD50 of 2.5 g/kg BW which is rather high (US EPA, 1992). This is a dose that is not easily reached with the uptake of foods. However, recently a possible activation metabolic pathway was identified during which the HMF becomes a highly reactive sulfuric acid ester and it was shown that this metabolite can react with the DNA (Glatt and Sommer, 2006; Glatt et al., 2011).

The legal limit for HMF in honey was set due to restricted processing conditions to 40 mg/kg. This limit is not based on toxicological reasons (EC Directive 74/409/EEC; UK Honey Regulations 2003). In the fair trade standards a quality grading system is used to produce honey with a HMF content as low as possible suggesting values of below 20 mg/kg (Fairtrade Standards 2005).

FORMATION OF HMF

The formation of HMF in the foods depends mainly on the presence of precursors (primarily glucose, fructose, amino acids) the temperature and the pH. Especially lower pH values – that means acidic foods – can form high amounts of HMF. It is not only the high processing temperature but also long storage times contribute to high HMF concentrations as it was shown for Madeira wines. Within the storage of 15 years the HMF content rises from very low concentrations up to 250 to 600 mg/L (Murkovic, unpublished). The contribution of HMF to the aroma of any food product is negligible which means that – if it is eliminated – the product quality would not change. However, there are currently no concepts available that would contribute to a mitigation of HMF.

ANALYSIS OF HMF

Similar to acrylamide HMF is water soluble and can be extracted with water. However, to reduce a solubilisation of proteins a mixture of methanol or acetonitril with water is used. Normally, the concentration of HMF is so high that the extract has to be diluted before analysis by RP-HPLC. A gradient of methanol and water is used for elution and due to the high concentrations and good absorption at 280 nm UV detection can be used. If the matrix is complex or the concentrations in the extract are low HMF can be derivatized with dinitro-phenyl-hydrazine which can be detected at 400 nm or with

good sensitivity by mass spectrometry (Murkovic, unpublished results; Jöbstl et al., 2010).

CONCENTRATION OF HMF IN FOODS AND DIETARY EXPOSURE

HMF is a substance that can occur in rather high concentrations. A selection of foods containing high amounts of HMF is given in Table 1. The concentrations are up to several grams per kg in some selected foods. Especially roasting of coffee and substitutes, heat processing and long storage can lead to extremely high concentrations. In a small study Husoy and co-workers (2008) showed that the intake of coffee, dried fruits, honey and alcoholic beverages contributed most to the exposure to HMF.

Table 1 - Foods rich in HMF

Food	HMF content (mg/kg)	Reference
Coffee	100-1,900	a, b
Coffee (instant)	90-4,100	a, c
Chicory	200-22,500	a
Malt	100-6,300	a
Barley	100-1,200	a
Jam	5.5-1,200	a, b
Breakfast cereals	6.9-240	a
Dried fruits	25-2,900	b, c
Vinegar balsamic	190-3,400	a, d
Sweet wines (Port, Sherry, Madeira)	10-650	d

a: extracted from Capuano and Fogliano, 2011; b: Murkovic and Pichler, 2006; c: Husoy et al, 2008; d: Murkovic unpublished

CONCLUSION

Foods prepared with conventional cooking methods contain HAs that are mutagenic and carcinogenic as well. These substances are found ubiquitous but in relevant amounts only in strongly heated meat and fish. Because of the well-established risk of cancer it is necessary to reduce the exposure to HAs. However, the precursors are present in all types of meat, which results in a low exposition of every human. Although it is impossible to prevent the HA formation completely a reduction of the uptake of these carcinogenic substances can be achieved by different ways: (1) intensive frying of meat and fish should be avoided, (2) if burnt food is served, these burnt parts should be removed and not eaten (3) the content of HAs is not so high if the food is prepared in the microwave oven (4) local overheating can be avoided if the meat is wrapped in aluminium foil (5) marinating and the use of antioxidant spices reduces the content of HAs (6) the consumption of meat and fish should be reduced. If these points are taken into account when meat or fish is prepared the risk of cancer due to heterocyclic aromatic amines can be reduced. The selection of raw material is not only an issue for reducing the formation of acrylamide but also for exposure to HCAs.

All three types of compounds that are presented here can contribute to the dietary cancer risk. As these are side products of aroma forming reactions it is difficult to avoid the formation without changing the characteristics of the product. The reduction of the exposure can be achieved by changing the cooking methods and reduce the heating to avoid over cooking. In some cases it can also be recommended to reduce the uptake of certain foods.

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IZVOD

FORMIRANJE KANCEROGENIH MATERIJA TOKOM ZAGREVANJA HRANE

Formiranje neki izabranih kancerogena tokom kuvanja je povezano sa nekim vrstama hrane. Heterociklični amini se uglavnom nalaze u mesu i ribi koja se kuva na temperaturama iznad 150 stepeni. Akrilamidi formirani u hrani sadrže asparagin i smanjenje šećera koji su prisutni u krompiru, žitaricama i sličnim proizvodima. Za formiranje akrilamida potrebne su visoke temperature koje se dešavaju tokom pečenja i prženja. Za razliku od predhodna dva tipa supstanci, 5-hidroksimetil-2-furfural se formira na nižim temperaturama, čak i tokom čuvanja namirnica iz ugljenih hidrata u prisustvu amino kiselina, ili iz fruktoze direktno u toku dehidratacije. Pošto su sva ova jedinjenja nusproizvodi i formiraju se u toku reakcije, teško je da se pronađu načini da se smanji sadržaj u hrani bez promene karakteristika prehrambenih proizvoda. Pravilan izbor sirovina i dobro kontrolisano kuvanje/obrada namirnica, u kombinaciji sa smanjenjem uzimanja nekih visoko zagađenih namirnica, može da nam pruži mogućnost da se smanji izloženost uticaju štetnih sastojaka hrane.

Ključe reči: *kancerogene materije, kontaminirana hrana, grejanje*

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