

## THE EFFECT OF DIFFERENT PREPARATION METHODS ON THE FORMATION OF HETEROCYCLIC AMINES

**Monika Gibis, Alexander Schoch and Albert Fischer**

Department of Meat Technology, Institute of Food Technology, University of Hohenheim, 70593 Stuttgart, Germany,  
e-mail: gibis@uni-hohenheim.de

### Background

Epidemiological studies have shown that diet is an important factor in the occurrence of human cancer (DOLL and PETO, 1981). In the search for possible relationships between diet and cancer, the highly mutagenic Heterocyclic Amines (HAs) present in cooked foods were found by Japanese scientists 20 years ago (SUGIMURA et al., 1977). Since their discovery, about 20 heterocyclic amines have been identified in cooked foods like fried, broiled or cooked meat or fish (FELTON and KNIZE, 1990). These mutagens are probably formed from creatinine, aldehydes, and Maillard reaction products such as pyrazines, pyridines and aldehydes. The International Agency on Cancer Research has classified several HAs as possible human carcinogens and has recommended a reduction in exposure to these substances (IARC, 1993). The important influences on the formation of HAs are the temperature and the heating time (ARVIDSSON et al., 1997). However, the heat transfer to the surface of the product and the mass transport of the precursors outwards to the crust of meat also affect the formation of HAs.

### Objectives

The aim of this study was to examine the influence of different preparation methods on the content of the most important precursor - creatine/creatinine - and the concentration of HAs in the investigated beef patties.

### Materials and Methods

**Preparation of beef patties:** Beef, roughly desinewed and defatted, was coarsely minced through a 3 mm plate. 1.2 % salt was added to the minced beef separately. The raw material was mixed with the blender. 80 g  $\pm$  1 g of the material were formed into beef patties with a special mold for hamburgers.

**Heat treatments:** The core and the surface temperatures of each treatment were monitored with a data logger (Ahlborn, Holzkirchen, D).

1. Convection oven: The patties were laid on tin foil which was coated with oil. The patties were baked to the core temperatures of 75°C, 85°C and 95°C corresponding to 12, 15 and 18 minutes with heat convection in a convection oven (Wiesheu Wiwa, Lenzkirchen, D) at a temperature of 230° C. Every 60 seconds they were treated with steam for 1 second.

2. Deep fryer: The patties were deep fried to the core temperatures of 75°C, 85°C and 95°C corresponding to 5, 7 and 9 minutes with frying fat at a temperature of 190°C.

3. Double contact grill (Nevada, Neumärker, D): The patties were laid on tin foil which was coated with oil. The patties were fried on both sides simultaneously to the core temperature of 75°C, 85°C and 95°C corresponding to 5, 7 and 9 min. The two grill plates were preheated at the temperature of 230°C.

4. Grill plate: The patties were laid on tin foil which was coated with oil. They were fried on a grill plate with the temperature of 230°C. After turning the patties over, the patties were fried to the core temperature of 75°C, 85°C and 95°C corresponding to 12, 16 and 20 min.

**Determination of HAs:** The method included the polar and apolar HAs. The method of HPLC analysis with some modifications was based on the method described by GROSS and GRÜTER (1992). The peaks of HAs, also Norharman and Harman, in samples were identified by comparing the retention times and UV-spectra with standards.

**Determination of creatine/creatinine:** Creatin/creatinine were determined enzymatically according to the Boehringer test kit instruction (ANOMYM, 1989).

**Determination of weight loss during cooking:** The patties were weighed raw and 1 h after the heat treatments.

### Results and Discussions:

HAs were found in all patties, but in very different concentrations. Especially MeIQx (2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline), Norharman and Harman were found in nearly all the patties (Fig.1). The  $\beta$ -carbolines Norharman and Harman are not mutagenic in the Ames Test, but have been shown to be co-mutagenic (HATCH, 1986). PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine) and 4,8-DiMeIQx (2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline) were determined only from the grill plate and double contact grill with core temperatures of 85°C and 95°C. The MeIQx content of all heat treatments increased with greater degree of doneness. The highest concentration of MeIQx (1.01 ng/g) was determined from the grill plate with turning over of the beef patties. This method is comparable to the household frying in a frying pan. The frying on both sides simultaneously to the same core temperature showed a reduction of 75 %, 65 % and 40 % respectively to the content of MeIQx. This preparation resulted in a shorter cooking time and a lower weight loss of 26 %, 38 % and 37 % respectively during cooking than the preparation with one grill plate and turning over of the patties (Fig. 2).

The lowest concentrations of MeIQx were found in patties which were deep fried. This method, however, had the highest concentrations of the precursor creatinine (Fig. 3) and similar high weight loss as the preparation with one grill plate. Either HAs were not formed in the same amount with this preparation or perhaps they are to be found in the deep-fry fat. In comparison to the other heating treatments the preparation with the convection oven produced the lowest concentration of the precursor creatinine and a very low concentration of MeIQx. Although this method had the longest cooking time and the same cooking temperature as the grill plate and the double contact grill, the heat transfer of the convection air with steam to the surface of the product was lower than the other materials. The presence of steam, which effected the heat transfer, decreased the surface temperature of the products. This could be due to the fact that in comparison to iron and oil, convection air is a bad heat conductor. This physical effect also plays an

important role in the formation of the HAs. Also SHINHA et al. (1998) reported that pan fried steaks had a higher content of MeIQx than oven broiled steaks. Another cooking method, oven-roasting, produced fewer HAs than pan frying (SKOG et al., 1997) which due to less efficient heat transfer in the air than when the product has direct contact with a frying pan. The heat and the mass transport in meat during frying is very complex. Inside a zone of water evaporation was formed. This zone moves inwards to the middle of the patties. Through the protein denaturation, water and juices are released and move outwards. The juice is important for the mass transport of the water-soluble precursors such as creatinine and Maillard reaction products to the crust. The single-sided cooking methods on a grill plate as well as in a pan have the disadvantage that the pores of the beef patties are only closed on one side, so that the juice escapes from the top surface until the patties were turned over. This effect can lead to weight loss and to increased formation of MeIQx, Norharman and Harman. Double side grilling, frying in a convection oven or deep frying have the advantage that the patties are cooked simultaneously at both sides.

# Conclusions

The analysis of food products made by different preparation methods is important for the formation of HAs because there is widespread human exposure to these carcinogenic compounds. The concentrations of HAs vary in different methods of food preparation. Appropriate heat treatment can reduce the formation of HAs and the individual human exposure to these compounds.

# Pertinent literature

ANONYM (1989): Methoden der biochemischen Analytik und Lebensmittelanalytik. Böhringer, Mannheim, Germany; ARVIDSSON, P., BOEKEL M.A.J.S. van, SKOG, K. and M. JÄGERSTAD (1997): J. Food Sci. 62, 911-916; DOLL, R. and PETO, R (1981): J. Natl. Cancer Inst. 66, 1191-1308; FELTON, J., and KNIZE M.G.(1990): In: Handbook of Experimental Pharmacology, ed. C. S. Copper and P. L. Grover, pp. 471-502. Springer Verlag, Berlin; GROSS, G.A. and A. GRÜTER (1992): J. Chromatogr. 592, 271-278; HATCH, F. T. (1986): Environ. Health Perspect. 67, 93-107; IARC (1993): IARC Monographs on the evaluation of carcinogenic risks to humans. No.56. Pp. 165-242. International Agency for Research on Cancer; SINHA, R.; ROTHMANN, N.; SALMON, C. P., KNIZE M. G., BROWN, E. D., SWANSON, C. A., RHODES, D., ROSSI, S., FELTON, J. S. and O. A. LEVANDER, O. A.(1998): Food Chem. Toxicology 36, 279-287; SKOG, K., AUGUSTSSON K., STEINECK, G., STENBERG, M., JÄGERSTAD, M. (1997): Food Chem. Toxicol. 35, 555-565; SUGIMURA, T., NAGAO, M., KAWACHI, T., HONDA, M., YAHAGI T., SEINO, Y., SATO, S., MATSUKARA, M., MATSUSHIMA, T., SHIRAI, A., SAWAMURA, M. and MATSUMOTO, H. (1977): In: Origins of Humans Cancer, pp.1561-1577, Cold Spring Harbour Laboratory, New York

# Acknowledgements

The authors thank Mrs K. Hoppert, Mrs S. Lasta and Mrs N. Mannowetz for their technical assistance.

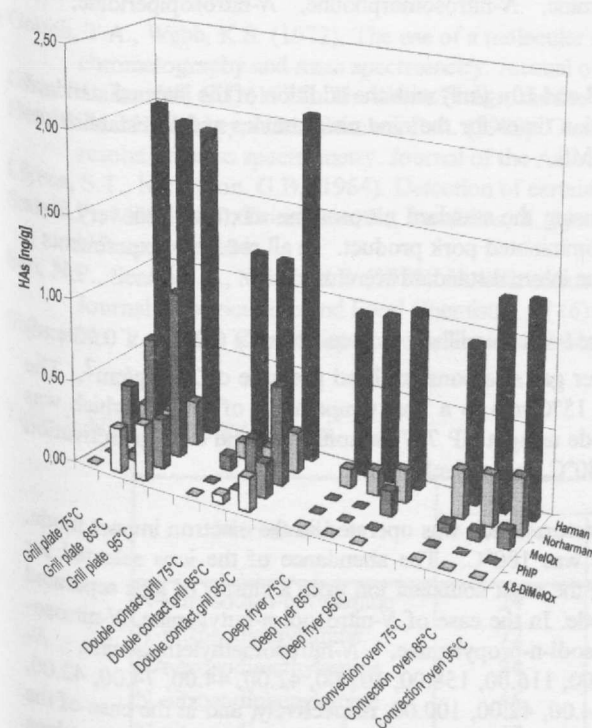


Fig. 1: Concentration of HAs in beef patties cooked by different preparation methods to three core temperatures

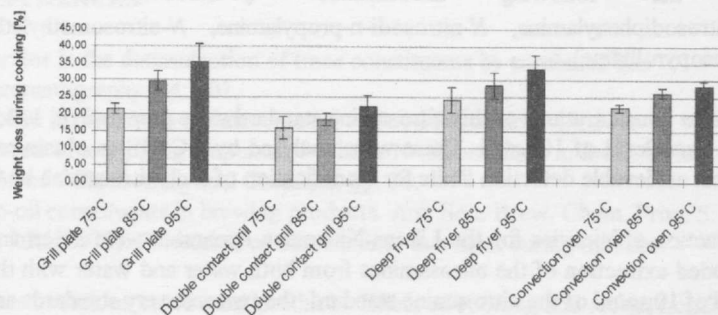


Fig. 2: Weight loss during cooking by different preparation methods and degree of donness

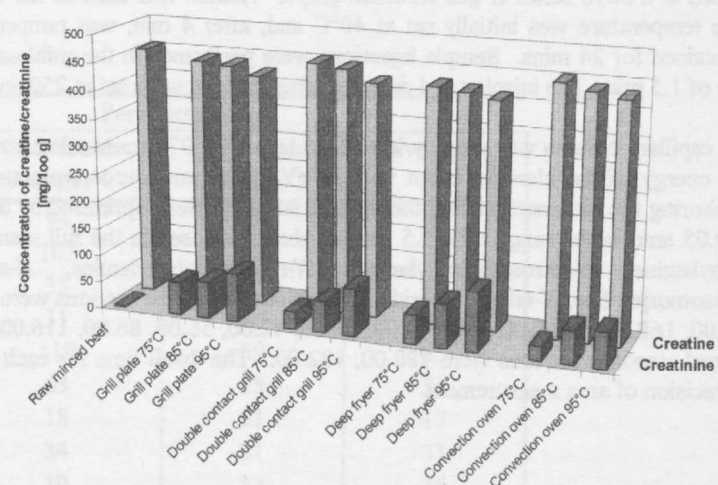


Fig. 3: Concentration of creatine and creatinine in beef patties cooked by different preparation methods to different core temperatures