HETEROCYCLIC AROMATIC AMINES IN HEATED MEAT

Jonas Damasius, Petras Rimantas Venskutonis

Kaunas University of Technology, Department of Food Technology,
Radvilenu pl. 19, 50254 Kaunas, Lithuania, e-mail: jonas.damasius@stud.ktu.lt

Abstract

It is well–known that cooking of various meats at high temperatures results in the formation of compounds that are not present in uncooked meats. Heterocyclic aromatic amines (HCAs) are an important class of heat-produced compounds. They were found to be mutagenic and carcinogenic for animals and even for humans. The aim of this study was to measure the amount of HCAs in beef during very long time and high temperature cooking. After sample extraction from the model system by using Chromabond XTR and Chromabond PS-H^+ cartridges the concentrations of HCAs[1] were separated by HPLC and measured by a mass spectrometry using mass selective detection mode. Six HCAs (IQ; MeIQ; 4,8-DiMeIQx; MeAαC; Trp-P-1; PhIP) were identified and quantified in the heated beef samples. The concentrations of these HCAs varied from undetectable levels to 50.4 ng g^{-1}. MeAαC and Trp-P-1 were present in heated beef extracts at the highest concentrations, while the values for the amino imidazoazaarenes were lower. PhIP which is one of the most abundant HCAs in conventionally heated meat was found at rather high concentration.

Key words: heterocyclic aromatic amines, heated beef, amino imidazoazarenes, amino carbolines

Introduction

Heterocyclic aromatic amines (HCAs) are formed during the cooking of meat (Keating *et al.*, 1999). Since the discovery of heterocyclic aromatic amines in foods a large number of scientific publications have been published on this subject until nowadays. Generally, there are two classes of HCAs: amino imidazoazarenes and amino carbolines. The amino-imidazo part of the amino imidazoazaarene molecule is formed from creatine (creatinine), while other substitutes come from Strecker degradation products (pyridines or pyrazines), which are formed in the Maillard reaction between sugars and amino acids. Amino-carbolines are formed during pyrolysis of amino acids or proteins at higher than 300 °C temperature. Both of these classes contain more than 20 different compounds. Some of them are strong mutagens and carcinogens as it was observed in the experiments with animals (Sugimura, 2002); moreover, some study models showed how HCAs may influence the formation of human cancer (Felton, 1995).

There are a lot of factors influencing the formation of HCA in meat. However, it seems that the most important factors are four: food composition, temperature, time and contact with heat source. Therefore, the increase of temperature and/or heating time may result in higher HAs concentrations. The aim of this study was to measure the amount of HCAs in beef during very long time and high temperature cooking.

Materials and Methods

Chemicals and materials

All HCAs references were purchased from Toronto Research Chemicals (Toronto, Canada); acetic acid, acetonitrile, methanol (all HPLC grade) and diethylene glycol were from Sigma-

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Abbreviations of HCAs:

PhIP	1-Methyl-6-phenyl-1H-imidazo[4,5-b]pyridin-2-amine CAS No.: 105650-23-5;
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;
4,8-DiMeIQx	2-Amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline CAS No.: 95896-78-9;
Trp-P-1	3-Amino-1,4-dimethyl-5H-pyrido[4,3-b]indole, acetate CAS No.: 68808-54-8;
Trp-P-2	3-Amino-1-methyl-5H-pyrido[4,3-b]indole CAS No.: 72254-58-1;
AαC	2-amino-9H-pyrido[2,3-b]indole CAS No.: 26148-68-5;
MeAαC	2-amino-3-methyl-9H-pyrido[2,3-b]indole CAS No.: 68006-83-7;
Glu-P-1	2-amino-6-methyldipyrido[1,2-A:3',2'-D]imidazole, hydrochloride hydrate CAS No.:67730-11-4;
Glu-P-2	2-aminodipyrido[1,2-α:3',2-D]imidazole, hydrochloride CAS No.: 67730-10-3.

Aldrich (Germany); ethyl acetate from Chempur (Poland); sodium hydroxide, hydrochloric acid and ammoniumhydroxide (25%) from Merck (Darmstadt, Germany). Diatomaceous earth extraction Chromoband XTR (70 ml, 14500 mg, kieselguhr) and Chromoband PS-H+ (strong PS/DVB cation exchanger in H+ form) cartridges were provided by Macherey-Nagel (Duren, Germany).

Meat preparation and cooking

Beef was purchased from the local market. One kg of raw beef meat was homogenized, freeze-dried and stored in a freezer at -18 °C until use. One g of freeze-dried meat was heated with diethylene glycol (10 ml) in crucibles at 220 °C for 30 min using oven (grill with convection program). The crucibles with meat were immediately cooled on ice after heating.

Extraction of HCAs

The chromatographic separations of HCAs were performed using modified method (Messner & Murkovic, 2004). All samples were dissolved in 12 ml 1 M NaOH and homogenized for 30 min at 150 rpm. The alkaline solution was poured into Extrelut cartridges. Ethyl acetate (50 ml) was used as the extraction solvent and the eluate was passed into PS-H^+ cartridges. The cartridges were washed with 0.1 M HCl (2 ml) and MeOH (2 ml) and HCAs were eluted with 2 ml MeOH-concentrated ammonia (19/1, v/v). All samples were evaporated to dryness (using nitrogen) and extracts were dissolved in 100 mg methanol before measurement.

Identification and quantification of HCAs

All conditions were maintained similar as described previously (Messner & Murkovic, 2004). HCAs were identified and their amount was determined by HPLC-MS system using Waters ASSC-MS equipment (Waters, Milford, USA) equipped with Waters 1525 ASSC pump, Waters ZQ-2000 mass spectrometer on a reverse phase analytical column (Altima C18 5u, 150 mm, ID 2.1 mm). The data was analyzed using Mass Lynx 4.0 software (Micromas UR Ltd., England). Mass selective detector was equipped with an atmospheric pressure ionization electrospray (API-ES) using a fragmentation voltage of 45 V for positive ionization. Drying nitrogen was heated to 350 °C and the drying gas flow was 10 l/min.

The data were acquired in the selected ion mode for HCAs and calculated in the extract ion mode. The HCAs were quantified using calibration curve of each HCA in MeOH.

Results and Discussion

Preparation of beef samples, identification and quantification of HCAs were performed using new model system described elswhere (Messner & Murkovic, 2004). LC-MS chromatograms of the selected solutions of HCAs references in MeOH and beef meat samples heated for 30 min at 220 °C in crucibles are presented in Figure 1; six HCAs (IQ; MeIQ; 4,8-DiMeIQx; MeAαC; Trp-P-1; PhIP) were identified in beef samples.

LC-MS chromatograms of several solutions of HCAs references in MeOH (100 ng ml) and beef meat samples heated for 30 min at 220 °C in crucibles are presented in Figure 1.

The concentrations of HCAs in freeze dried beef samples are presented in Figure 2.

In general, the concentration of different HCA in meat varied from undetectable to 50.4 ng g^{-1}. MeAαC (50.4 ng g^{-1}) and Trp-P-1 (31.4 ng $^{-1}$) were present in highest amounts in beef extracts. It should be noted that unusually high amount of MeAαC was found in meat of our study comparing to some previously published results (Skog *et al.*, 1998; Toribio *et al*., 2000; Messner & Murkovic, 2004); the concentration of MeAαC in earlier studied real and/or model systems was more than 10 times lower. Comparatively small amounts of IQ (11.1 ng g^{-1}), MeIQ (14.3 ng g^{-1}) and 4.8-DiMeIQx (10.6 ng g^{-1}) were formed during thermal treatment of meat. PhIP which is one of the most abundant HCAs in conventionally heat treated meat was determined in our meat samples in a quite high concentration constituting 22.3 ng g^{-1}. It should be noted that this finding differs from the previously reported, when beef samples were heated using almost the same model system at the same time and temperature (Messner & Murkovic, 2004). It might be due, that in our experiment 10 times higher amounts of samples and reagents there were prepared and cooked.

Also, some role may play differences in a type of cooking oven as well as extraction cartridges, which were used in our and above mentioned study. Others available HCAs as a reference compounds were not found in cooked meat samples.

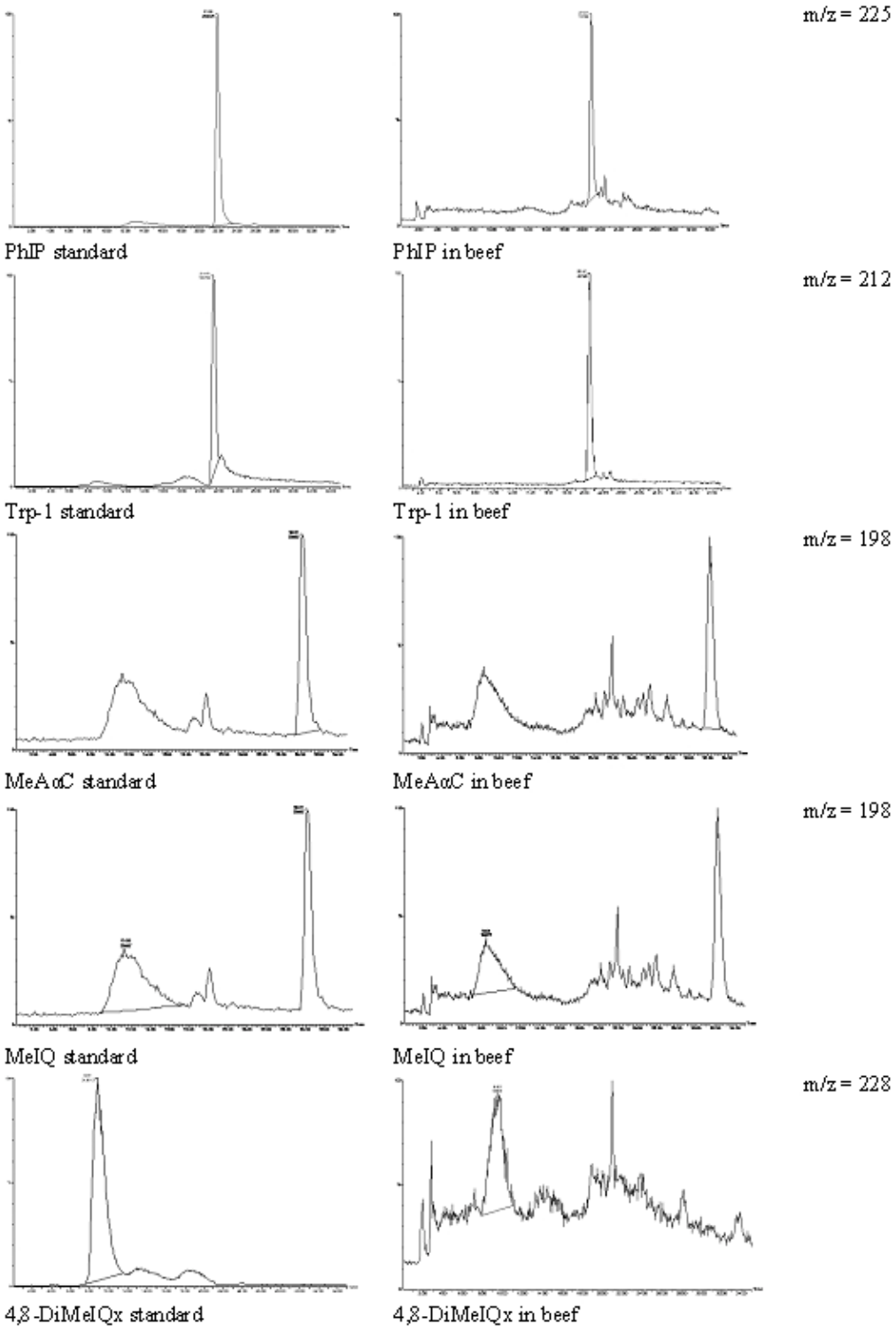

Figure 1. LC-MS chromatograms of several solutions of HCAs references in MeOH (100 ng/ml) and beef meat samples heated for 30 min at 220 °C in crucibles

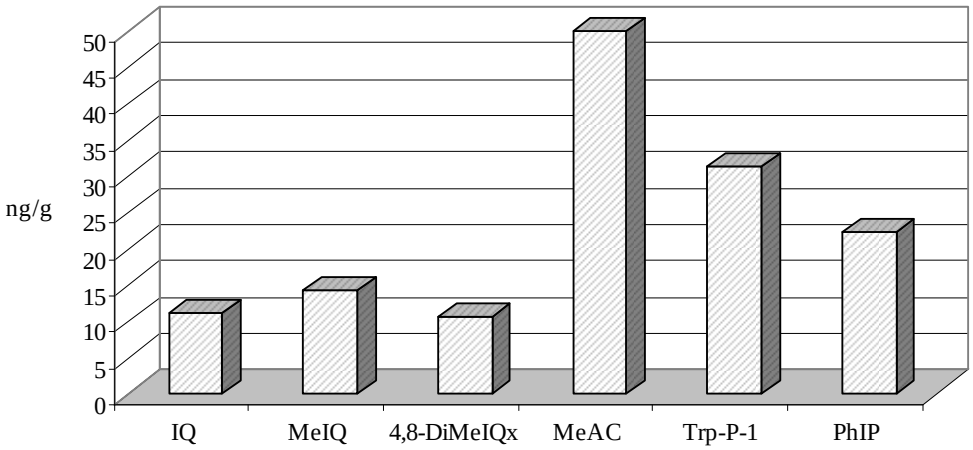

Figure 2. HCAs concentrations in freeze-dried beef samples after heating for 30 min at 220 °C temperature

The comparison of the data obtained on various HCAs indicates that there was a significant difference between the concentration of amino imidazoazaarenes and amino carbolines. The values for the amino imidazoazaarenes were lower. Most likely this finding can be explained by the fact that carbolines and their analogues usually form during treatment of meat at very high temperatures. Consequently, heating 1 g of meat for 30 min at 220 °C can be considered as a very long time and high temperature thermal treatment.

Conclusions

Five of six HCAs which were identified in beef samples possess strong mutagenic-carcinogenic properties. The concentrations of the identified HCAs in heated meat varied from undetectable levels to 50.4 ng g^{-1}. MeAαC and Trp-P-1 were present in heated beef extracts at the highest concentrations. The concentration of PhIP, which is one of the most abundant HCAs in cooked meat was also present at a quite high quantity. The values of IQ; MeIQ; 4,8-DiMeIQx in heated beef were lower, on average 12 ng g^{-1}. It can be concluded that higher amounts of amino-carbolines HCAs than amino imidazoazaarenes type HCAs are formed during long time and high temperature cooking of beef.

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