Searching for urinary biomarkers of exposure to heterocyclic amines (HCAs)

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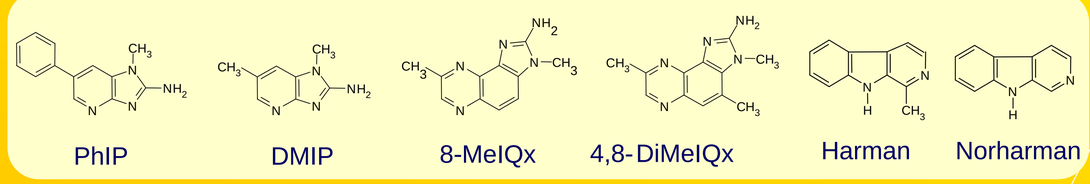
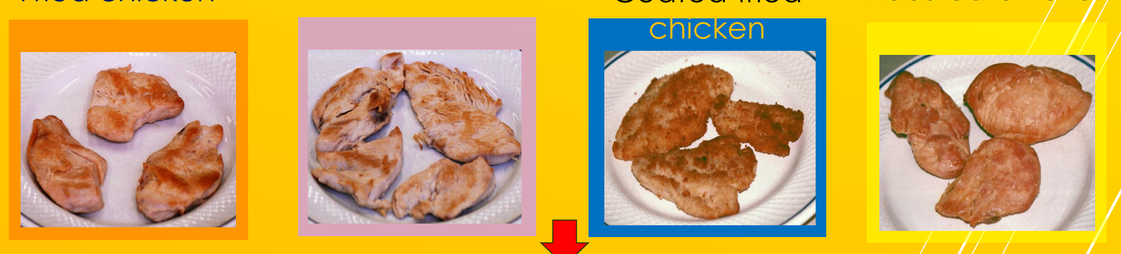
Introduction

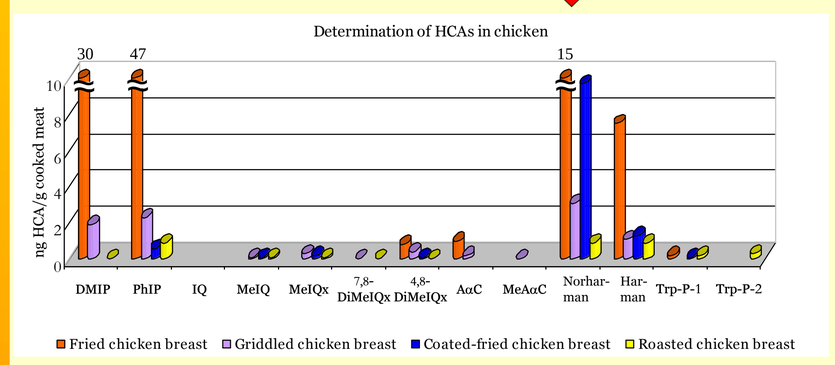
The Maillard reaction takes place in cooking processes and yields a wide range of compounds; the mutagenic heterocyclic amines (HCAs) among them. The formation of HCAs depends on the cooking conditions, such as temperature and time, and presence of chemical precursors (sugars, creatine/creatinine, amino acids) or inhibitors [1]. With the aim to find biomarkers of exposure to HCAs in accessible tissue, metabolites from HCAs have been determined in the urine of a group of 8 volunteers who ate a controlled meal consisting of fried chicken (120g) cooked for 6 min at 180 °C and gravy made from the frying drippings. The controlled meal accounted for a total of 6 µg of HCAs. The urine was analysed by a method based on liquid-phase microextraction and liquid chromatography-tandem mass spectrometry developed by our team [2].

Most abundant HCAs in cooked meat and fish



Fried chicken | Griddled chicken | Coated-fried chicken | Roasted chicken





Toxicology:

IARC: Grup 2A (possible human carcinogen): IQ

Grup 2B (probable human carcinogen): MeIQ, MeIQx, PhIP, AaC, MeAaC, Trp-P-1, Trp-P-2, Glu-P-2.

Materials and Methods

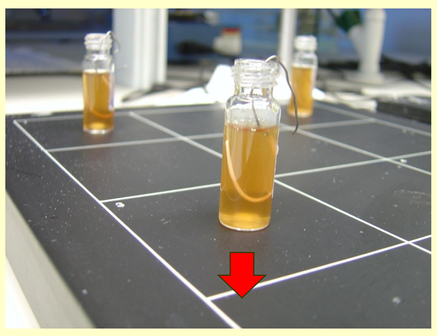
Controlled meal given to 8 volunteers

T: 182 °C; cooking time: 6min; Frying fat: smör-rapsolija; 36% cooking loss

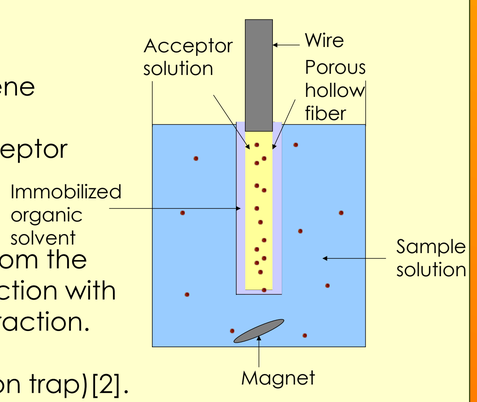
Collection of urine samples for 12h

The oxidation of metabolites was prevented by adding EDTA and propylgallate in the urine samples.

Analysis of HCAs metabolites in urine with extraction using hollow fibres [2]



Extraction conditions (urine samples) Polypropylene hollow fibre (pore size: 0.2 µm, ID: 600 µ m, wall thickness: 200 µm. Organic solvent: octanol. Acceptor solution: 0.5M H_2SO_4. Urine adjusted to pH 5.5.

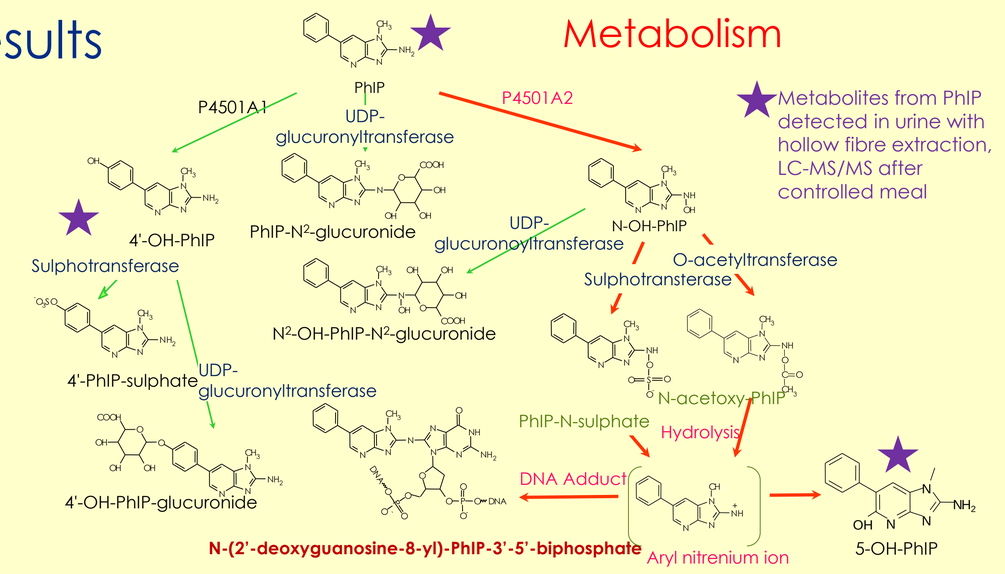


Analysis of HCAs in food: HCAs were extracted from the chicken using homogenisation with NaOH, extraction with ethyl acetate and clean-up with solid phase extraction.

Determination of HCAs : LC-MS/MS (LCQ Deca ion trap)[2].

Results

Metabolism



★ Metabolites from PhIP detected in urine with hollow fibre extraction, LC-MS/MS after controlled meal

Results

Quantification of HCAs in the controlled meal

HCA	ng/g in chicken	ng/g in gravy	Estimated intake of HCAs (ng) per person
PhIP	6.5±1.0	9.8±3.1	1020
4'-OH-PhIP	9.7±3.4	33.9±3.8	1969
DMIP	6.7±1.7	13.6±2.3	1130
MeIQx	2.3±0.6	3.8±0.7	373
4,8-DiMeIQx	0.3±0.1	<LOD	36
Harman	1.8±0.4	1.6±1.0	254
Norharman	6.5±0.9	1.8±1.0	831
Total			5613

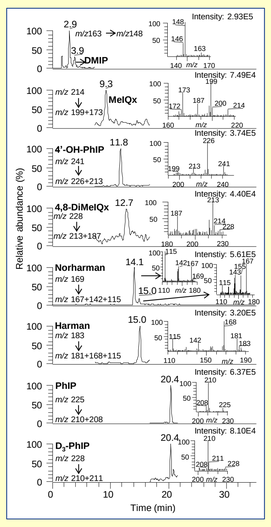
Quantification of the PhIP in urine samples from volunteers.

Subject	Urine weight (g)	PhIP injested (ng)	PhIP (pg/g urine)	PhIP (% dose)
man1	776	1024	15.6±7.2	1.2
man 2	406	1019	35.9±12.6	1.4
man 3	475	1013	29.7±1.0	1.4
man 4	623	1019	23.3±6.3	1.4
woman1	300	1020	59.4±5.7	1.7
woman 2	523	1012	30.4±7.2	1.6
woman3	663	1025	31.8±2.6	2.1
woman4	799	1025	11.7±3.2	0.9

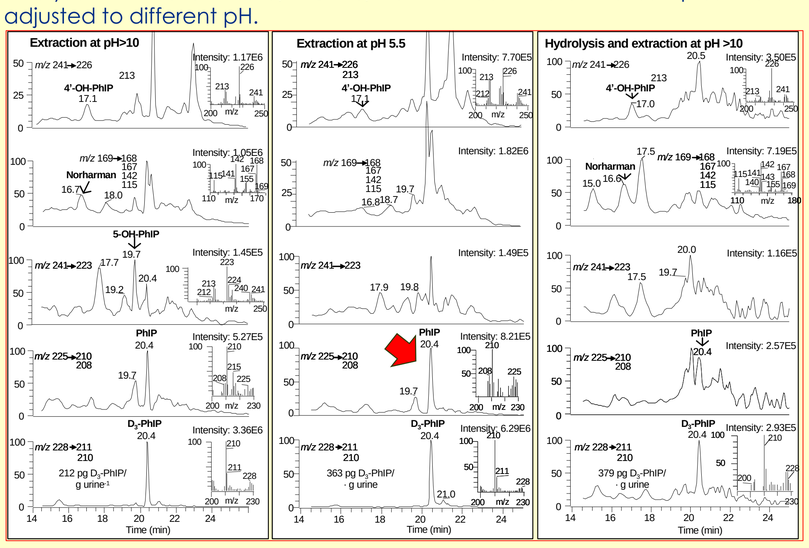
LOD 0.02 ng/g chicken or gravy

LOD 2 pg/g urine sample

HCAs purified from the controlled meal.



Chromatograms of HCAs and metabolites extracted from the urine sample from one of the volunteers obtained 12h after the controlled meal. Analysis with hollow fibre/ LC-MS/MS method where the samples were adjusted to different pH.



Conclusions

- The developed analytical method based on micro-extraction of HCAs using hollow fibres and detection with LC-MS/MS has made possible to detect the study compounds in urine after the intake of a portion of non-overcooked fried chicken.
- The selectivity of the extraction is affected by the pH of the sample. PhIP was quantified in all the urine samples collected when the pH was 5.5. When pH was adjusted at 12, the selectivity of the extraction was lower.
- A narrow variation of one of the HCAs, PhIP, was observed among the volunteers: 0.91-2.1% of the initial dose. This let us propose PhIP as potential biomarker [3]. The volunteers had different sex, origin, age and yet the amount of PhIP quantified in urine was similar. This may be an evidence that the activity of the enzymes involved in the metabolism of PhIP could be similar among the volunteers.
- Norharman, 4'-OH-PhIP, 5-OH-PhIP, PhIP and new HCA-related metabolites have been identified in the urine samples in this work. 5-OH-PhIP had been proposed as biomarker of the genotoxic dose of PhIP by the authors [4]. These findings will help to establish a relation between HCAs and cancer.

References

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2. R. Busquets, J. Å. Jönsson, H. Frandsen, L. Puignou, M.T. Galceran, K. Skog. Hollow-fibre supported liquid membrane extraction and LC-MS/MS detection for the analysis of heterocyclic amines in urine samples. Mol. Nutr. Food Res. 53 (2009)1496-1504.

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4. H. Frandsen, H. Frederiksen, J. Alexander. 2-Amino-1-methyl-6-(5-hydroxy-)phenylimidazo[4,5-*b*]pyridine (5-OH-PhIP), a biomarker for the genotoxic dose of the heterocyclic amine 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP). Food Chem. 40 (2002)1125-1130.