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# MITIGATING HETEROCYCLIC AMINES FORMATION IN BEEF MEAT USING SPECIFIC PROCESS CONDITIONS

A. Kondjoyan<sup>1</sup>, S. Chevolleau<sup>2,3</sup>, S. Portanguen<sup>1</sup>, P. Ikonic<sup>4</sup>, S. Clerjon<sup>1</sup>, L. Debrauwer<sup>2,3</sup>

<sup>1</sup> QuaPA UR 370, INRA, 63122 Saint Genes Champanelle, France

<sup>2</sup> INRA, UMR1331 TOXALIM, Research Center in Food Toxicology, MetaToul-AXIOM Platform, F-31027 Toulouse, France.

<sup>3</sup> Université de Toulouse, INPT, UPS, UMR1331, F-31062 Toulouse, France.

<sup>4</sup> Institute of Food Technology, University of Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia.

**Abstract – Development of a crust during meat cooking is an important element for consumer. It is the place of the formation of color and flavor. However, it is also where process induced toxicants can be formed. In this paper, the development of the colored crust was followed at the surface of a meat cylinder subjected to a hot air jet. The thickness of the crust and the Heterocyclic Aromatic Amines content were measured under this situation which mimics the roasting of beef meat. Results prove that it is possible to maintain the aspect of roasted meat while reducing dramatically the HAs content.**

**Key Words – Carcinogens, Cooking, Mitigation, Roasting, Control**

## Abbreviations:

4,8-diMeIQx: 2-amino-3,4,8-trimethyl-imidazo[4,5-f]quinoxaline

AcC: 2-amino-9H-pyrido[2,3-b]indole

DMIP : 2-amino-1,6-dimethylimidazo[4,5-b] pyridine

Glu-P-1: 2-Amino-6-methyldipyrdo[1,2-a:3',2'-d]imidazole

Glu-P-2: 2-Aminodipyrdo[1,2-a:3',2'-d]imidazole

HAs : Heterocyclic Aromatic Amine

IQ: 2-amino-3-methyl-imidazo[4,5-f]quinoline

IQx: 2-amino-3-methyl-imidazo[4,5-f]quinoxaline

MeIQ: 2-amino-3,4-dimethyl-imidazo[4,5-f]quinoline

MeAcC: 2-amino-3-methyl-9H-pyrido[2,3-b]indole

MeIQx: 2-amino-3,8-dimethyl-imidazo[4,5-f]quinoxaline

PEEK: PolyEther Ether Ketone

PhIP: 2-amino-1-methyl-6-phenyl-imidazo-[4,5-b]pyridine

TriMeIQx: 2-amino-3,4,7,8-tetramethyl-3H-imidazo[4,5-f]quinoxaline

Trp-P-1: 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole

Trp-P-2: 3-amino-1-methyl-5H-pyrido[4,3-b]indole

TSP-d4: 3-(trimethylsilyl)-2,2',3,3'-tetrauteropropionic acid

## I. INTRODUCTION

Heterocyclic Aromatic Amines (HAs) are formed in trace amounts in cooked muscles. The potential carcinogenic properties of these amines have been demonstrated in numerous studies [1] [2]. Thus, it is important to understand HAs formation and to reduce their content during roasting and grilling. HAs formation rate increases with temperature and depends on the time-temperature history of the surface region: the “crust” where they are formed. Crust development also leads to the specific roasted and grilled flavour, and colour, seek by consumer. In this study, HAs formation and crust development have been analyzed under an accurately controlled situation which mimics the roasting of beef meat; the aim being to find some conditions which maintain the visual aspect of roast meat while mitigating the formation of HAs.

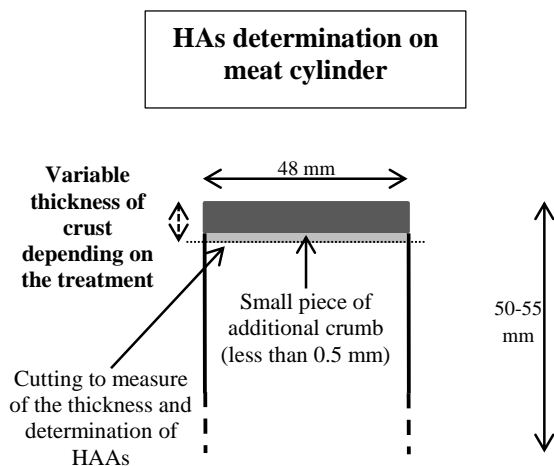
## II. MATERIALS AND METHODS

### *Sample preparation and crust measurements*

*Longissimus thoracis* muscles were taken from carcasses of 18-month-old heifers immediately after slaughter. The muscles were cut into big pieces, aged for 12 days under vacuum-packed conditions at 4 °C, then frozen and stored at -20 °C. Before experiments, a cylinder of meat 48 mm in diameter and 50 to 55 mm in height was cut from the previous big pieces of muscles. Experiments were performed using an open hot air jet system which enables the IR measurement of the temperature at the surface of the meat, and using a specific device which partially compensates heat shrinkage of the sample and enabled the thermocouple measurement of undersurface meat temperatures [3]. Heating was stopped by sliding the support beneath 45-55 m.s<sup>-1</sup> jet flow of cold air (temperature

3-5 °C) produced by a Ranque-Hilsch vortex pipe. After cooling, the meat cylinder was removed from the support. Some of the cylinders were used to analyze crust formation and structure; while the top of other cylinders which contained the colored crust plus an additional small portion of “non-brown-colored” meat was used to quantify HAAs (Fig. 1). To measure the crust thickness a set of pictures of crust area was taken using a camera ( $\times 10$  zoom) associated to binoculars ( $\times 1.6$  or  $\times 2$  magnification) and a  $100 \times 0.1 = 10$ -mm scale (PYSER-SGI, UK). In a first step, GIMP 2.6 software was run on the image to accurately separate the brown-colored crust area from the “non-brown-colored” area following the irregular border. In a second step, a calibration of lengths was done using the pictures of the reference length taken in each image to avoid any difference in image resolution. In a third step, average crust thickness and its standard deviation were calculated from all the acquisitions obtained on different portions of crust.

Figure 1. Sample preparation.



*Identification and quantification of HAAs in the colored crust*

Thirteen different HAAs (IQ, IQx, MeIQ, MeIQx, 4,8-diMe-IQx, A $\alpha$ C, MeA $\alpha$ C, Trp-P-1, Trp-P-2, PhIP, Glu-P1, Glu-P2, DMIP) were determined by U-HPLC-APCI-MS/MS according to a method previously developed for chicken meat by LC-APCI-MS/MS [4] and adapted afterwards for the analysis of beef meat samples [5] [6].

### III. RESULTS AND DISCUSSION

Colored crust thicknesses determined from binocular images increased linearly with time whatever the jet temperature [3]. When jet temperature was 124 °C, colored crust thickness was only 0.2 and 0.6 mm after 20 and 90 min of treatment, respectively. When jet temperature is 210 °C, crust was thicker than 1.0 mm after 20 min of treatment and reaches more than 5.0 mm after 90 min of treatment [3].

Four of the HAAs, namely Trp-P-1 Trp-P-2, A $\alpha$ C and MeA $\alpha$ C, were never detected in the colored crust or only as traces while quantities close to our limit of detection were detected for Glu-P-1 and IQ in the most drastic roasting conditions (210 °C, 90 min). The concentrations of the other HAAs measured in the crust area of the meat cylinder are given in Table 1. When the temperature was less or equal to 158 °C, only MeIQx and PhIP were formed in significant quantities, with concentrations below 20 ng.g<sup>-1</sup> DM for MeIQx and below 5 ng.g<sup>-1</sup> DM for PhIP. As soon as the temperature reached 192 °C, all the HAAs are formed significantly except MeIQ and Glu-P-2. PhIP and MeIQx concentrations began to be important and reached 157 ng.g<sup>-1</sup> and 220 ng.g<sup>-1</sup> for these two HAAs at 210 °C respectively. Only the concentration of MeIQ remained low whatever the temperature conditions.

Table 1. Detailed HAAs content in the developing meat crust (for 30-40 min cooking time) given as nanograms of compound per gram of freeze-dried product (including the crust and the piece of additional non-brown-colored area).

Roasting conditions (°C)	124	158	192	210

<b>Crust thickness (mm)</b>	0.19	0.72	1.42	2.59
<b>MeIQx</b>	19.3	13.7	88.3	219.9
<b>IQx</b>	1.7	nd	5.1	11.6
<b>DiMeIQx</b>	nd	traces	5.2	14.0
<b>PhiP</b>	4.8	1.2	21.0	156.6
<b>MeIQ</b>	traces	traces	nd	1.8
<b>DMIP</b>	nd	traces	13.0	48.7
<b>Glu-P-2</b>	nd	traces	traces	24.8

During roasting, HAs concentrate in the upper part of the crust while other parts of the meat piece are insufficiently heated to generate HAs (temperature below 124 °C). The effect of temperature on the formation of HAs clearly appears in Table 1, showing that MeIQx and PhiP begin to form in very weak amounts only for 158 °C. In Table 1, the HAs concentrations are expressed by weight of dry matter of crust while crust mass has to be taken into account to determine the consumer exposure to HAs. Thus, crust thicknesses measured after the different heat treatments by [3] are also reported in Table 1. The air jet heat treatment at 158 °C applied during 40 min leads to HAs contents which can be considered as very small in the colored crust which is also thin. Thus, the real mass content of HAs can be considered as negligible while the surface of the meat is perceived visually as being crusted. Thus, some heating conditions can be found to maintain the aspect of roast beef meat while reducing dramatically HAs content in meat cylinders subjected to hot air jets. However, this time-temperature conditions were observed under the present specific air jet situations and has to be confirmed for other type of cooking equipments. More generally, the evolution of the surface and the under-surface temperatures depend on the air-flow velocity and on the heat exchanged by radiation, which are actually equipment dependent.

#### IV. CONCLUSION

Heating conditions were found to maintain the aspect of roast beef meat while reducing dramatically HAs content in beef meat cylinder subjected to hot air jets. However, other heat-mass transfers studies are needed to validate such conditions for other types of roasting and grilling equipments. Sensory analysis studies and consumer acceptance inquiries are still also needed to analyze the impact of the heating conditions on meat color and flavor.

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