Temperature regime and formation of carcinogenic heterocyclic aromatic amines

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Abstract. The effects of thermal treatment conditions on the 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) and 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx) levels formed in pork samples of different matrices (pork steak vs. pork mince) were studied. Oven air temperature oscillations above the set-point temperature of 200 °C were produced using an on-off controller. Oscillations that reached 1.2 °C amplitude showed formation of one-fifth to one-tenth the PhIP levels in the pork steak and mince compared to the maximum air temperature oscillations that produced temperatures of up to 211.5 °C. The MeIQx levels in pork steaks were not significantly affected by the temperature oscillations, while they were doubled in pork mince. To reduce the levels of these heterocyclic aromatic amines, an oven with precise temperature control is significantly more important than other options during the cooking of pork.

1. Introduction

Domestic cooking processes are rarely studied, although they can drastically modify the quality of the heated foods, in terms of nutritional and sensory characteristics [8, 5, 11]. Despite the advantages of the cooking process, one prime concern is the formation of heterocyclic aromatic amines (HAAs). The choice of appropriate methods for thermal treatment of meat to minimise formation of HAAs is a particularly important issue. Specifically, severe cooking methods with temperatures well above 200 °C (e.g., frying, grilling, barbecuing), and/or prolonged cooking times can lead to excessive formation of HAAs [2, 6, 14].

During oven thermal treatment of meat of different sizes and muscle types, two particular aspects need to be considered: the mass transfer that drives the crust formation, and the water loss by evaporation and by protein denaturation-contraction [10].

To evaluate the performance of oven-baking appliances used in domestic cooking, their characterisation in terms of oscillations in the amplitude and frequency above the set-point temperature (i.e., compliance with and oscillations above the set-point temperature) appears to be more informative than standard air-temperature measurements [1, 4, 5, 16].

To summarise the aims of the present study, it should be kept in mind that even slight variations in oven air temperature above the set-point temperature (i.e., elevated air temperatures) or greater oscillations in the air temperature can lead to increased meat-surface desiccation and overheating, and consequently might also result in increased formation of HAAs. Based on this concept, we investigated the levels of the HAAs 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) and 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx) formed in pork steaks and in minced pork thermally treated in an oven. This domestic cooking appliance (i.e., oven) was chosen because they are commonly used in homes, and there appears to be no data available regarding the HAAs that can be formed as a result of set-point temperature oscillations during the heat treatment of pork meat in different forms.
2. Materials and methods

2.1. Experimental design
Fresh pork loins of standard quality were cut into ten steaks with a thickness of approximately 5 cm. These steaks were vacuum packed, immediately frozen, and kept at -35 °C. Before the analyses, the frozen steaks intended for cooking (heating) the following day were thawed overnight at 4 °C. These thawed steaks were then cut into two similar halves, to provide smaller steaks with a thickness of 2.5 cm (mean weight, 115 ±18 g). Then, one half of each smaller steak was minced and formed into a patty-shaped sample (mean weight, 80 ±1 g; using a Petri dish). The (smaller) steaks and mince were assigned as randomised pairs for thermal treatment in an oven using 10 replicate procedures, thus eliminating any effects of meat location in the muscle.

The oven used (Gorenje+ GO978X) had a defined volume of 75 L, energy consumption at venting of 0.71 kWh, and under conventional conditions of 0.94 kWh, with a connected load of 3.4 kW, a triple glazed oven door with double heat deflector, and oven cooling. The oven had modified firmware, which allowed the setting of temperature oscillations above the set-point temperature of 200 °C, but it should be noted that the average temperature in all thermal treatments was equal (205 °C). For thermal treatment of meat, the difference between the maximum and the set-point temperature is important.

The corresponding steak and mince samples were placed in the preheated oven together and thermally treated for 40 min at 200 °C (i.e., the optimal time to prepared a 400 g pork roast). Different temperature oscillations were applied during the cooking times. The actual air temperature was measured throughout these heating procedures using a spear-pointed temperature probe (Testo 177-T4, coupled to a stainless steel class 1 probe) that was inserted into the mid-point of the oven. Each individual experiment was repeated three times.

2.2. Calculation of cooking loss
The cooking losses from the meat samples were expressed as percentage weight loss.

2.3 Determination of heterocyclic aromatic amines
After 24 h of cooling at ±1 °C, HAA levels were determined in the roasted pork steaks and minced pork. HAAs levels were determined by the method described by Santos et al. (15), with minor modifications, as described by Polak et al. [12].

For liquid chromatography-mass spectrometry (LC-MS) analysis, the cooked meat samples were cleaned up using solid-phase extraction (Oasis, MCX 60 mg columns). The LC-MS analyses (Agilent 1100 system) were performed on an analytical column operated under reverse phase conditions (semi-micro TSKgel ODS-80Ts column, 5 μm, 250 mm x 2 mm i.d.; Cat. N° 18151; Tosoh Bioscience LLC, Japan) at 25 °C. The separation was performed at a flow rate of 0.3 mL/min by gradient elution with 20 mmol/L ammonium formate (Cat. N° 09739; Fluka) at pH 3.2 as solvent A, and acetonitrile (Cat. N° 1.00030; Merck) as solvent B. The injection volume was 10 μL, and the internal standard was 4,7,8-TriMeIQx. The HAAs were identified and quantified according to their retention times and the spectra from reference samples of known concentrations run under the same conditions.

2.4 Data analysis
The experimental data were evaluated statistically using the SAS/STAT programme. Basic statistical parameters were calculated using the MEANS procedure. The data were tested for normal distributions. The effects of sample matrix (pork steak, minced pork) on PhIP and MeIQx levels were evaluated using paired t-tests, and the main effects of temperature oscillations above the set-point temperature of 200 °C (1.2 °C to 11.5 °C) and repetitions/animal (1-3) were evaluated using the general linear model procedures. The means for the oscillation amplitudes were obtained using Duncan’s procedure, and were compared at the 5% probability level.
Table 1. Effects of temperature oscillations on heterocyclic aromatic amine (PhIP and MeIQx; µg/kg thermally treated sample) levels in pork steaks and minced pork and the differences in PhIP and MeIQx levels relative to the thermally treated samples. Data are mean ± standard deviation, where indicated (n = 120).

| Oscillation amplitude (°C) | PhIP | MeIQx |
|----------------------------|------|-------|
|                            | Pork steak | Minced pork | Pork steak | Minced pork |
|                            | $P_S$ | $P_S$ | $P_S$ | $P_S$ |
| 1.2                        | 3.88±0.80$^{a}$ | 2.37±0.25$^{b}$ | 0.062 | 5.02±2.79$^{a}$ | 1.71±0.99$^{Db}$ | 0.064 |
| 1.5                        | 4.73±0.27$^{a}$ | 3.97±0.55$^{ab}$ | 0.052 | 4.88±0.87 | 3.65±0.72$^{bc}$ | 0.217 |
| 1.6                        | 7.27±0.58$^{a}$ | 4.66±0.61$^{ab}$ | ≤0.001 | 8.31±1.16$^{a}$ | 4.51±1.07$^{bc}$ | 0.027 |
| 1.7                        | 7.28±0.36$^{a}$ | 7.50±0.48$^{ab}$ | 0.569 | 6.93±1.64$^{a}$ | 3.05±0.72$^{b}$ | 0.007 |
| 2.7                        | 9.71±0.24$^{ab}$ | 11.49±0.52$^{c}$ | 0.005 | 5.91±2.15$^{a}$ | 3.27±1.27$^{bc}$ | 0.099 |
| 4.1                        | 11.09±0.64$^{a}$ | 11.05±0.97$^{c}$ | 0.950 | 6.49±1.02$^{a}$ | 3.60±1.53$^{bc}$ | 0.021 |
| 5.5                        | 10.86±1.38$^{a}$ | 9.92±0.87$^{c}$ | 0.300 | 5.59±1.43$^{a}$ | 3.02±1.43$^{b}$ | 0.018 |
| 7.8                        | 15.96±2.08$^{a}$ | 11.21±0.85$^{c}$ | 0.031 | 9.23±3.74$^{a}$ | 5.82±1.37$^{bc}$ | 0.094 |
| 9.3                        | 18.58±1.02$^{b}$ | 15.61±2.55$^{c}$ | 0.037 | 6.67±3.85$^{a}$ | 2.91±1.69$^{b}$ | 0.041 |
| 11.5                       | 20.78±1.83$^{a}$ | 23.04±2.28$^{a}$ | 0.327 | 7.16±1.93$^{a}$ | 2.68±1.27$^{bc}$ | 0.004 |
| $P_P$                      | ≤0.001 | ≤0.001 | 0.117 | 0.001 |
| Overall                    | 11.01±5.60$^{a}$ | 10.08±5.97$^{b}$ | 0.027 | 6.62±2.42$^{a}$ | 3.33±1.56$^{b}$ | ≤0.001 |

PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine; MeIQx, 2-amino-3,8-dimethylimidazo[4,5-f]quinoline; $P_S$, Statistical probability of sample matrix effect; $P_P$, Statistical probability of oscillation amplitude effect; $P_S$ values with different superscript capital letters within columns (°) differ significantly (i.e., significance of differences between different oscillation amplitudes); $P_P$ values with different superscript small letters within columns (°) differ significantly (i.e., significance of differences between pork steak and minced pork).

The samples contained PhIP at 10.55 µg/kg and MeIQx at 4.98 µg/kg in terms of the thermally treated samples (cooked weight), and when calculated relative to the fresh samples, in terms of the wet weights (fresh weight), these were PhIP at 5.37 µg/kg and MeIQx at 2.55 µg/kg. The pork steaks contained PhIP at 11.01±5.60 µg/kg cooked weight and MeIQx at 6.62±2.52 µg/kg cooked weight (Table 1), levels that were considerably higher than those reported earlier [12], i.e., PhIP at 0.77 µg/kg cooked meat and MeIQx at 0.90 µg/kg cooked meat for their 120-g steaks that were grilled on two-plated grill at 220 °C to an internal temperature of 70 °C. Similarly, [13] reported PhIP at 2.29 µg/kg.
and MeIQx at 0.23 µg/kg in pork roast (650-680 g) that was cooked at an oven temperature of 177 ºC and an internal temperature of 71 ºC. In comparison to the present data [19] reported similar PhIP at 13.12 µg/kg and MeIQx at 7.59 µg/kg for pork loins slices with a thickness of 2 cm, and pan-fried at a surface temperature of 204 ºC to an internal core temperature of 77 ºC. For the minced pork in the present study, the HAAs were measured as PhIP at 10.08±5.97 µg/kg cooked weight and MeIQx at 3.33±1.56 µg/kg cooked weight (Table 1).

Despite the relatively large variability, there were some significant differences seen for the PhIP and MeIQx levels. Thus, the effects of the sample matrix (i.e., pork steak, pork mince) and the effects of the temperature oscillations were statistically significant (P ≤ 0.05) for both PhIP and MeIQx, as both cooked weight and fresh weight (Tables 1, 2).

The highest PhIP levels were seen in the pork steak and minced pork treated under the highest temperature oscillations (11.5 ºC). PhIP levels were intermediate in the samples with oscillations of 9.3 ºC, and 7.8 ºC, followed by the samples with oscillations of 2.7 ºC, 4.1 ºC and 5.5 ºC, and finally were lowest with the lowest oscillation amplitudes of 1.7 ºC and less. These lower levels of PhIP for oscillation amplitudes below 1.7 ºC (pork steak and mince, 2.37-7.50 µg/kg cooked weight) as compared to those for the highest oscillations of 11.5 ºC (pork steak and mince, 20.78-23.04 µg/kg cooked weight) are thus due solely to the approximately 10 ºC difference in the oscillation amplitudes above the set-point temperature of 200 ºC.

Increasing HAAs levels are produced with increasing cooking temperatures (150-225 ºC), and PhIP levels are generally higher than MeIQx levels at higher temperatures [17]. In the present study with the pork steaks, MeIQx was the most abundant HAA at temperature oscillation amplitudes below 1.6 ºC (4.88-8.31 µg/kg cooked weight), although the PhIP levels increased to higher levels than MeIQx when temperature oscillation amplitudes were above 2.7 ºC (9.71-20.78 µg/kg cooked weight). However, for the pork mince, PhIP was the most abundant HAA regardless of temperature oscillations (Tables 1, 2).

As a consequence of the oscillations above the set-point temperature to give a 12 ºC increase, both the formation and degradation of MeIQx was expected. This was seen for in the pork steaks, where the balance of the MeIQx levels was essentially independent of the temperature oscillations (P=0.117), and remained between 4.88 µg/kg and 9.23 µg/kg cooked weight. On the contrary, in the pork mince, the MeIQx levels depended on the temperature oscillations during the thermal treatment (P=0.001), with the highest levels seen at the oscillation amplitude of 7.8 ºC.

Kondjoyan et al. [9] emphasised the meaning of the crust, the thin area close to the surface of the meat that thickens during grilling and roasting. The HAA levels in whole meat samples depend on the gradients of temperature and water content/water activity in the thickening crust [18]. As suggested later by Kondjoyan et al. [10], crust formation at the surface of small meat pieces will differ from that in larger meat pieces due to the different thickening of the crust and the migration of the HAA precursors. On this basis, the question arises as to whether there are differences in HAA levels between pork steaks and pork mince. Generally, in comparison to pork steak, the pork mince showed lower PhIP levels, although these differences were only statistically significant under some of the temperature oscillation conditions (Table 1; 1.6, 2.7, 7.8, 9.3 ºC).

Table 2. Effects of temperature oscillations on heterocyclic aromatic amine (PhIP and MeIQx; µg/kg raw sample; wet weight) levels in pork steaks and minced pork and the differences in PhIP and MeIQx levels relative to the raw pork samples. Data are means±standard deviation, where indicated (n = 120).

| Oscillation amplitude (ºC) | PhIP              |           | MeIQx              |           |
|---------------------------|-------------------|-----------|-------------------|-----------|
|                           | Pork steak        | Minced pork | Ps                 | Pork steak | Minced pork | Ps                 |
| 1.2                       | 2.11±0.46<sup>a</sup> | 1.15±0.12<sup>b</sup> | 0.045             | 2.66±1.34<sup>a</sup> | 0.84±0.50<sup>b</sup> | 0.044             |
| 1.5                       | 2.46±0.12<sup>b</sup> | 1.92±0.20<sup>b</sup> | 0.023             | 2.53±0.40  | 1.78±0.40<sup>bc</sup> | 0.152             |
The data in Table 2 for the HAA levels in thermally treated pork expressed on a raw (i.e., fresh weight) basis can also be used in combination with dietary assessments to estimate the exposure to HAAs from the consumption of pork. This is because the temperature oscillations also affect the weight loss during the meat cooking, so for realistic evaluation, the data must also be defined for the raw meat. Here, the conclusions based on these data expressed as cooked weight and fresh weight are essentially the same, although the data tend to be less variable based on the fresh weights than for the thermally treated meats.

4. Conclusions
The potential carcinogenicity of red and processed meat has been evaluated according to the presence of well-known/suspected carcinogenic compounds such as N-nitroso-compounds, polycyclic aromatic hydrocarbons and HAAs [3, 7]. In particular for domestic cooking of red meat, there is clearly a lack of information on the potential carcinogen compound levels and the variability of the heating conditions according to the quality of the cooking appliance used. Our findings help to address this deficiency. The present study clearly shows that talking about the recommended intake of meat in terms of these carcinogen compounds (PhIP and MeIQx) without emphasis on the preparation and appliance parameters used is essentially illusory, if not factually incorrect. Here, we have clearly proven the appropriate heating conditions and the quality of the appliance/oven used are important for producing healthier oven-roasted meats containing lower levels of the HAAs studied. Moreover, less healthy meats will result if they are prepared with a poorly regulated domestic oven.

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