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AUTHORS: Alain Kondjoyan, Sylvie Chevolleau, Stéphane Portanguen, Jérôme Molina, Predrag Ikonic, Sylvie Clerjon, Laurent Debrauwer

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Relation between crust development and heterocyclic aromatic amine formation when air-roasting a meat cylinder

Kondjoyan, Alain [1†], Chevolleau, Sylvie [2, 3], Portanguen, Stéphane[1], Molina, Jérôme[2,3], Ikonic, Predrag [4], Clerjon, Sylvie [1], Debrauwer, Laurent [2,3]

[1]UR370 Qualité des Produits Animaux, INRA, F-63122 Saint Genès Champanelle, France.
[2]INRA, UMR1331 TOXALIM, Research Center in Food Toxicology, MetaToul-AXIOM Platform, F-31027 Toulouse, France.
[3]Université de Toulouse, INPT, UPS, UMR1331, F-31062 Toulouse, France.
[4]Institute of Food Technology, University of Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia.

Abstract

The meat crust that develops during cooking is desired by consumers for its organoleptic properties, but it is also where heterocyclic aromatic amines (HAs) are formed. Here we measured HAs formation during the development of a colored crust on the surface of a beef meat piece. HAs formation was lower in the crust than previously measured in meat slices subjected to the same air jet conditions. This difference is explained by a lower average temperature in the colored crust than in the meat slices. Temperature effects can also explain why colored crust failed to reproduce the plateauing and decrease in HAs content observed in meat slices. We observed a decrease in creatine content from the center of the meat piece to the crust area. In terms of the implications for practice, specific heating conditions can be found to maintain a roast beef meat aspect while dramatically reducing HAs content.

Key words: Crust, Heterocyclic amines, Heat, Juice, Transfer, Beef, Hot air.

Highlights:

HA formation and degradation differ between roast beef crust and meat slices;
Creatine content decreases from the center of the meat piece to the crust;
HA content can be dramatically reduced without losing a 'roasted' look.

[†] Corresponding author: UR370 Qualité des Produits Animaux, INRA, 63122 Saint-Genès-Champanelle, France, Tel: +33 (0)4 73 62 44 92 – Fax: +33 (0)4 73 62 40 89, email: alain.kondjoyan@inra.clermont.fr

Abbreviations:

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

$AcONH_4$: Ammonium acetate

ACN: Acetonitrile

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

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

FDP: Freeze-Dried Product

Glu-P-1: 2-amino-6-methyldipyrido[1,2-*a*:3',2'-*d*]imidazole

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

HAs: Heterocyclic Aromatic Amines

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

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

IR: Infrared

LD: *Longissimus dorsi*

LL: *Longissimus lomborum*

LT: *Longissimus thoracis*

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

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

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

MeOH: Methanol

MRM : Multiple Reaction Monitoring

PAH: Polycyclic Aromatic Hydrocarbons

PEEK: PolyEther Ether Ketone

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

SM: *Semimembranosus*

TB: *Triceps brachei*

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

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

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

TSP-d_4: 3-(trimethylsilyl)-2,2',3,3'-tetradeuteropropionic acid

1. Introduction

Cooking meat forms trace amounts of heterocyclic aromatic amines (HAs). The effects of HAs on human health have been widely investigated, and while the literature is not unanimous on the associations between HAs and human cancers, their carcinogenic effects have been proved on animals and on cell lines (Ollberding, Wilkens, Henderson, Kolonel, & Le Marchand, 2012; Jamin et al., 2013). Moreover, even if the carcinogenic risks of moderate consumption of well-done meat appear low (Nagao, 1999; Ollberding et al., 2012), there are synergetic effects to consider. For example, a synergistic effect has been identified with Polycyclic Aromatic Hydrocarbons (PAH) / PhIP mixtures on cell lines, with an increase of the formation of DNA adducts derived from PhIP (Jamin et al., 2013). Thus, understanding and reducing HAs formation during cooking remains an important concern for consumer health.

HAs formation rate increases with temperature and depends on the time-temperature history of the surface region, i.e. the "crust", where HAs are formed. Crust development also generates the specific roasted and grilled flavours and colour desired by consumers. Several kinetic models of HAs formation and degradation have been developed in liquid and meat juice systems (Arvidsson, Van Boekel, Skog, & Jägerstad, 1997; Arvidsson, van Boekel, Skog, Solyakov, & Jägerstad, 1999) as well as in meat slices subjected to jets of superheated steam or dry air in the 150-250°C temperature range (Kondjoyan et al., 2010a, b). Concentrations of four of the studied HAs, i.e. IQx, MeIQx, 4,8-DiMeIQx and PhIP, follows regular kinetic patterns. HAs degradation was studied in a meat juice model system at 200 and 225 °C (Ardvidsson et al., 1999). In meat slices, HAs formation increases with treatment time and temperature during the first 10 min of treatment (Kondjoyan et al., 2010a, b), after which HAs concentration either plateaus or decreases at temperatures ≥200 °C. The increase in the formation of 4 HAs (MeIQx, PhIP, norharman and harman) in function of the temperature and the cooking time has also been shown recently by Gibis, Kruwinnus & Weiss (2015) in fried bacon. Nevertheless, it is difficult to compare the concentrations of HAs because of the differences in composition between the relatively lean beef and pork rather rich in fat. No degradation was observed at 175 °C. At 200-250 °C, HAs degradation is dependent on type of HAs and gas humidity. In our previous experiments in meat slices, temperature was uniform throughout the meat, and the whole slice can be considered

'crust'. Moreover, the experiments were limited to 20 min. These parameters are key factors when comparing HAs formation in liquid and solid media. However, the situation is different during the development of a crust on the surface of a meat piece during grilling or roasting. In these practical conditions, strong temperature and water gradients the surface of the meat cut lead to crust formation and thickening.

Creatine and creatinine are known to be common precursors to the formation of all HAs (Nagao, 1999; Bordas, Moyano, Puignou, & Galceran, 2004; Gibis & Weiss, 2010). For example, half of the carbons of PhIP come from creatine and the other half from phenylalanine (Zöchling & Murkovic, 2002). Creatine and creatinine also play a major role the formation of IQx and its related derivatives (Borgen, Solyakov & Skog, 2001). In meat, the concentration of creatine is about tenfold the concentration of creatinine. Creatine dehydration occurs spontaneously and non-enzymatically in the muscle to form creatinine (Purchas, Rutherfurd, Pearce, Vather & Wilkinson, 2004), but this reaction is strongly promoted by increasing meat temperature (Mora, Sentandreu & Toldra, 2008), which means cooking meat decreases creatine/creatinine ratio (Harris, Lowe, Warnes & Orme, 1997; Mora et al., 2008). Model systems can be used to modulate the reaction precursors to determine their effect on the formation of different HAs, which cannot be done in meat pieces (Kondjoyan et al., 2010a, b; Bordas et al., 2004; Arvidsson et al., 1997, 1999).

This paper aims at connecting HAs formation to the mechanisms which are responsible for crust formation. A special device and procedure were used to accurately control a thermal treatment mimicking the roasting of beef meat (Portanguen, Ikonic, Clerjon, & Kondjoyan, 2014). Creatine content was also measured from the center of the meat piece out to the area of crust formation to assess its potential correlation with the formation of HAs such as PhIP or IQx. Specific heating conditions were also looked for in order to maintain the roasted aspect on the surface of beef meat while reducing HAs under a situation which mimics the baking of beef meat in a forced-convection oven.

2. Materials and Methods

2.1. Sample preparations, heat treatments, and thickness 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, then frozen and stored at −20 °C. Thermal Treatment and temperature measurement were performed using: (1) an open jet system which enables IR measurement of surface temperature, and (2) a specific device that partially compensates for the heat shrinkage of the sample.

A cylinder of meat, 48 mm in diameter and 50–55 mm in height, was accurately cut from big pieces of the previous muscles, following the procedure detailed in Kondjoyan et al. (2010a, b). The meat cylinder was placed in a support formed by a bigger hollow cylinder made in PEEK®. The cylindrical meat sample was pushed by a spring fixed to a disc located at the bottom of the device onto a grid fixed by a lid at the top of the device. Spring contraction was adjusted using four screws located under the disc. The support was then placed under the center of an open jet system. During heat treatment, air temperature at the outlet was either 160, 190, 225, or 260 °C, and distance (d) between sample surface and pipe outlet was set at 36 mm. Actual temperatures reached on the surface of the meat sample are given in Table 1. Heating was stopped by sliding the support beneath a 45– 55 m s^{-1} jet flow of cold air (temperature 3–5 °C) produced by a Ranque–Hilsch vortex tube. Meat surface temperature was measured continuously throughout thermal treatment using a calibrated IR pyrometer (Kondjoyan et al. 2010a, b). During heating and cooling, the temperatures of the air jet impacting the meat were measured using a thermocouple fixed to the PEEK® cylinder and set 2 mm above the meat surface. After cooling, the meat cylinder was removed from the support taking every precaution not to tear away any crust while withdrawing the surface grid. Samples were used to analyse crust thickness and HAs content.

To measure the thickness of the colored crust, the top of one half of the meat sample was cut perpendicularly to the cylinder axis such that the thickness of the slice was equal to the thickness of the brown-colored crust plus a further 2– 3 mm of “non-brown-

colored" crust. A set of pictures of crust area was taken using a camera (×10 zoom) associated to binoculars (×1.6 or ×2 magnification) and a 100×0.1=10-mm scale (PYSER-SGI, UK). The thickness of the colored area was measured visually from the binocular images. Measurements were taken in at least five crust locations to obtain repeatable average and standard deviation values. In a first step, GIMP2.6 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 (specific programs developed using Matlab 7.0® and its image processing toolbox).

2.2. Quantification of creatine

Creatine content was measured on meat cylinders exposed to the hot air jet at 192 °C for either 20 or 60 min. After the treatment, four 5 mm-thick slices of meat were cut from the top of the cylinder perpendicularly to jet direction (Fig. 1, right). Creatine was measured by NMR in raw and cooked meat samples. Microsamples (0.2 g) were ground in $Na_2HPO_4/NaH_2PO_4{\cdot}12H_2O$ buffer at pH 5.0 (Acros Organics, Geel, Belgium). After 12 h of rest, the solution was filtered and centrifuged for 10 min at 14,500 g at room temperature. Then, a 0.6 mL aliquot of the supernatant was then assayed by ^{1}H-NMR spectroscopy. Creatine was characterized by two characteristic resonance signals: a singlet resonance at 3.03 ppm (CH_3) and a singlet resonance at 3.92 ppm (CH_2). The integration of these two signals was proportional to the number of protons. Quantifications were made with respect to a closed capillary containing TSP-d4 (3-(trimethylsilyl)-2,2',3,3'-tetradeuteropropionic acid, Sigma-Aldrich, Saint-Quentin-Fallavier, France) measured relative to a 1.76 mmol.L^{-1} standard solution of creatine.

2.3 Identification and quantification of HAs in the colored crust

Thirteen different HAs (IQ, IQx, MeIQ, MeIQx, 4,8-DiMe-IQx, AαC, MeAαC, Trp-P-1, Trp-P-2, PhIP, Glu-P1, Glu-P2, DMIP) were determined by UHPLC-APCI-MS/MS based on an LC-APCI-MS/MS method developed for chicken meat (Chevolleau,

Touzet, Jamin, Tulliez & Debrauwer, 2007) and since adapted for analyzing beef meat samples (Kondjoyan, et al., 2010a, b). Meat samples were treated as described in previous papers (Kondjoyan, et al., 2010a, b).

Briefly, 1 g of lyophilized beef meat sampled from the whole cooked sample was first treated with 1 M NaOH. The internal standard (TriMeIQx) was added at this stage at a concentration of 50 pg/µL. After liquid/liquid extraction (methylene chloride, Extrelut), purification by solid-phase extraction (Oasis MCX 60mg/3cc) and evaporation to dryness, the resulting residue was re-dissolved in 200 µL of the starting LC mobile phase. Separation was performed on an Accela 600 LC system (Thermo Fisher Scientific, Les Ulis, France), using a Hypersil GOLD C8 column (Thermo Fisher Scientific, 50 x 2.1 mm, 1.9 µm) with prefilter and a $AcONH_4$ (30 mM, pH5/ACN/MeOH) gradient elution. 5 µL of the final extract was injected, and separation was performed at 30 °C at a flowrate of 0.5 mL/min. MS detection was performed on a TSQ Vantage triple-stage quadrupole mass spectrometer (Thermo Fisher Scientific) using positive APCI ionization based on two specific transitions for each HA. Typical working parameters were as follows: discharge APCI current, 4 µA; nebulizer temperature, 420 °C; heated transfer capillary temperature, 250 °C; heated transfer capillary voltage, 35 V; sheath gas flow rate, 40 a.u.; auxiliary gas flow rate, 8 a.u. The performance of the optimized method was characterized in terms of linearity (r^2 > 0.99 between 1 and 1000 pg/µL), repeatability of retention times (RSD=1.5–2.0%, n = 6) and area ratios (RSD =5–10%, n = 6), LoD 0.015-0.05 ng/g) and LoQ (0.05–0.2 ng/g). No matrix effect (checked by post-column addition of the 13 HAs mixture) was observed. Each cooked meat sample was extracted and analyzed in triplicate. HA contents were expressed in nanograms of compound per gram of freeze-dried product.

2.4 Statistical analysis

All results in Table 3 were analyzed for normal distribution and subjected to an analysis of variance using HSD Tukey-test (α = 0.05) using the STATISTICA 12 Software (StatSoft France, France).

3. Results and discussion

3.1. Variations in creatine content in the meat cylinder during heating

In this study, results are expressed based on both dry matter (FDP) and as a function of weight of the roasted product. Results expressed on a FDP basis made it possible to track changes in creatine content regardless of juice loss variations. Results expressed on a mass of cooked product basis made it possible to compare our results with the literature. Figure 2 plots the profile of creatine content measured from the center of the meat piece to its surface when the meat cylinder was subjected to a jet of hot air at 192 °C for 60 min. A 20 min treatment led to a similar profile shape but the contents were different (*ca.* 20% less than in Figure 2; data not shown). At the center of the meat piece, creatine concentration was 501 mg/100 g cooked meat (1.85 g/100 g FDP), which matches to the concentration reported by Purchas, Busboom & Wilkinson (2006) for raw meat (Table 2). Gibis et al. (2015) measured the creatine concentration in bacon at different scales of time and temperature, the results show a decrease in creatine concentration with increasing temperature. This is also observed in present study as a function of depth in the meat, which is connected to the decrease of the temperature. Concentration expressed as a function of FDP decreases steadily towards the meat surface and then sharply once in the colored crust.

3.2. HAs content in the colored crust

U-HPLC-MS/MS was able to characterize and quantify 13 HAs with better performances than achievable using classical LC-MS/MS. Four of these HAs, namely Trp-P-1 Trp-P-2, AαC and MeAαC, were never detected in the coloured crust or only in trace amounts, while Glu-P-1 and IQ was detected in quantities close to our LoQ in the most drastic roasting conditions (210 °C, 90 min). Table 3 reports the concentrations of the other HAs measured in the surface area (including the colored crust plus the small portion of non-brown-colored area) of the meat cylinder.

The heat treatments can be divided into three categories according to HAs content. In the first category (lines 1, 2, 4 and 5 in Table 3), jet temperature and heating duration are less than or equal to 158 °C and 40 min, respectively. Only MeIQx and PhIP are

formed in more important quantities, with concentrations below 20 ng g^{-1} FDP for MeIQx and below 5 ng g^{-1} FDP for PhIP. The second category concerns the longest treatment at the lowest temperature (124 °C for 90 min) together with the shortest treatment at 192 °C (lines 3 and 6, table 3). In this case, all the HAs are formed in significant amounts except MeIQ and Glu-P-2. The MeIQx and PhIP concentrations measured were 35–40 and 5–15 ng g^{-1} FDP, respectively. In the third category, all the HAs are formed in high amounts, except MeIQ which remained under 3 ng g^{-1} FDP (lines 7 to 11 in table 3). In this category, two major HAs reach concentrations up to 416 ng g^{-1} FDP for MeIQx and to 255 ng g^{-1} FDP for PhIP.

The effect of cooking time on HAs concentration was studied from results reported in Table 3, using the HSD Tukey-test. Few effects of cooking time were not significant (values highlighted in grey in Table 3), concerning in particular the formation of PhIP at the lowest temperatures. Above 192 °C, time effect was found to be highly significant, except for the specific cases of IQx at 20 and 40 min at a temperature of 210 °C. The effect of time x temperature was also tested for the conditions corresponding to lines 1 to 3 and 9 to 11, showing shows a highly significant effect of the temperature (124 to 210 °C), of the cooking time (20, 40, 90 min) and of the two combined factors on the formation of MeIQx and PhIP.

3.3. Discussion

The HAs concentrations measured in this study within the colored crusts of small roasts can be compared to those measured in a thin slice of beef meat subjected to superheated steam or dry air jets (Kondjoyan et al., 2010a, b). The MeIQx and IQx concentrations within the colored crust of the small roasts were half the concentrations found in the slices at identical jet temperature and treatment duration. The proportions were more variable for the HAs known to form in a water activity-sensitive manner. The ratio of amounts of HAs formed in the colored crust *versus* the slice ranged from 2.0 to 9.0 for PhIP and from 0.8 to 2.0 for 4.8-DiMeIQx depending on the type of thermal treatment (dry air or superheated steam). The lower HAs content found in colored crust developed on the surface of the meat pieces compared to the thin slices can be attributed to either: (1) local differences in precursor concentrations, (2) differences in the “reactivity” of these precursors connected to the water activity in the food matrix, or (3) local differences in in-product temperature.

305

306 The first explanation cannot be connected with an initial difference in HAs precursors in
307 the raw meat, as all the experiments were led on the same beef muscle, i.e. *Longissimus*
308 *thoracis*. Thus, the local variation in precursors content can only be attributed to
309 differences due to their migration over the course of the experiment. In this study,
310 creatine concentration decreased from 1.85 g/100 g FDP at the middle of the meat piece
311 to 0.7 in the 2.5 mm just under the cylinder surface when the meat cylinder was roasted
312 at 192 °C for 60 min. The literature (Table 2) reports that creatine concentration
313 decreases down to 310 mg/100 g during grilling (Purchas et al., 2006) and 178 mg/100
314 g during frying (Gibis & Weiss, 2010) where the creatine likely mainly undergoes
315 dehydration, leading to an increase of creatinine concentration. Initial creatinine content
316 in raw meat from *Longissimus thoracis* muscle ranges from 8.4 to 11.4 mg/100 g
317 (Kondjoyan et al., 2010b). If the increase of creatinine is proportional to the decrease in
318 creatine, then creatinine content in the crust would be multiplied by about 30-fold
319 compared to the initial raw meat content. This could have important ramifications for
320 the formation of HAs, especially PhIP, since Bordas et al. (2004) reported that a 25-fold
321 increase in creatinine doubled the PhIP concentration in a meat flavor model system
322 subjected to a dry treatment at 200 °C for 30 min, whereas there was almost no effect on
323 PhIP when the model system was subjected to wet treatment at 170 °C for 2 hours.
324 Moreover, during Bordas' experiments, creatine content was held constant while
325 creatinine content varied, whereas under our more realistic conditions, creatine and
326 creatinine contents varied in the opposite pattern. Thus, in our set-up, the relative effect
327 of creatine and creatinine content on HAs formation on the surface of a piece of meat
328 strongly heated and dried during crust formation remains open to debate, especially
329 since this effect of creati(ni)ne can be mixed with that of the amino-acids. Indeed,
330 Bordas et al. (2004) also observed a strong effect of variation in amino acids content on
331 PhIP formation whereas we did not measure amino acids content in the crust area here.

332

333 The second explanation based on difference on precursor reactivity is supported by the
334 fact that in previous studies, it was observed that despite a higher amount of precursors,
335 IQx, MeIQx and 4.8-DiMeIQx formed at lower rates in meat slices than in meat juice
336 (Arvidsson et al., 1999, Kondjoyan et al., 2010a, b).

337

However, the third explanation seems the most likely here, since thin slices can be considered areas of high and homogeneous temperature whereas the colored crust featured sharp temperature gradients resulting in a lower mean temperature in the crust than in the slices. This difference stems directly from the development of the crust structure on the surface of meat pieces. We previously showed that crust is a complex structure displaying several distinct areas. The transition from non-crust to crust area begins at the evaporation front (Portanguen et al., 2014). Just beyond this front, liquid is replaced by vapour, but water content is still high. Therefore, water activity and temperature remain close to 1.00 and 100 °C, respectively. Water content then decreases toward the surface, which leads to areas of high-temperature gradients successively dominated by the formation of colored Maillard compounds and of HAs, respectively.

This study quantified HAs inside the coloured crust. A schematic drawing of the relative thickness of the different crust areas estimated after 70 minutes of treatment at 210 °C is given in Figure 3 (Portanguen et al., 2014). The area where the temperature is greater than or equal to 200 °C is about 0.5 mm deep and thus represents less than one third of the area where the in-product temperature is over 150 °C. This illustrates the extent of the temperature gradients near the surface of the meat pieces, and also explains why no plateau or decrease in HAs quantities were observed in the colored crust, contrary to our previous studies on meat slices where HAs plateauing or degradation were observed after just 10 minutes of heat treatment (Kondjoyan et al., 2010a, b). The slow HAs degradation that could occur in the 200 °C area just under the crust surface is likely overwhelmed by the strong HAs formation in the deeper area of the meat piece.

These observations tend to confirm that the major factor driving HAs formation and degradation is time–temperature. However, water activity was also found to play an important role during experiments on meat slices where HAs formation increased with decreased water activity due to the use of dry-air jets instead of superheated steam jets (Kondjoyan et al., 2010a, b). A better quantification of these different phenomena would require accurate heat and mass transfer modelling since measures are delicate due to the very thin areas which form the crust.

3.4. Mitigation of consumer exposure to HAs

372 During roasting, HAs concentrate in the upper part of the crust while other parts of the
373 meat piece do not get hot enough (i.e. below 124 °C) to start generating HAs. The effect
374 of time–temperature on HAs formation is clear in Table 3 showing that very low
375 amounts of MeIQx and PhIP only begin to form from 158 °C for 20 min. Indeed, the
376 quantities of MeIQx and PhIP measured at 158 °C for 20 min are approximately 35
377 times lower than the quantities measured at 158 °C for 40 min which, in turn, are still 3
378 to 10 times lower than at 192 °C for 20 min. The HAs concentrations expressed in Table
379 3 are per weight of crust based on FDP. Thus, crust mass also has to be factored in to
380 determine consumer exposure to HAs. We previously showed that the thickness of the
381 colored crust increases linearly with time (Portanguen et al., 2014). Note that Table 3
382 also gives the crust thicknesses measured after the different heat treatments. Crust
383 thicknesses greater than 3 mm were voluntarily discarded from analysis as not
384 representative of common cooking practices.
385
386 The air-jet heat treatment at 158 °C applied for 40 min leads to HAs contents that can be
387 considered very low in the thin colored crust. Thus, the real mass content of HAs can be
388 considered negligible even though the meat surface is visually perceived as 'crust'. In
389 terms of practical implications, specific heating conditions can be found to maintain a
390 roast beef meat aspect while dramatically reducing HAs content in the meat. Moreover,
391 the fact that crust thickness increases linearly with time raises prospects for controlling
392 crust development during the roasting process. However, this thickness increase was
393 observed under specific air-jet situations and has yet to be confirmed for other cooking
394 conditions. More generally, the time-course evolution of surface and under-surface
395 temperatures depends on air-flow velocity and the heat exchanged by radiation, which
396 vary from one set of equipment to another. Thus, specific thermal studies are needed to
397 further determine and control the optimal conditions to ensure safe meat roasting for
398 different types of cooking equipments.
399
400 **4. Conclusion**
401
402 Less HAs form in colored crusts developing on the surface of meat pieces than in meat
403 slices subjected to the same time–temperature conditions. This can be explained
404 considering the temperature of the whole slice is very rapidly reaches to air temperature
405 whereas the crust features a sharp temperature gradient resulting in a net lower mean

406 temperature of the crust than the surrounding air. Temperature gradients can also
407 explain why even at the longest treatment times and highest jet temperatures, the
408 colored crust failed to reproduce the plateauing and decrease in HAs content observed
409 in meat slices (Kondjoyan et al., 2010a, b). A decrease of creatine (which may result in
410 an increase of creatinine) was measured from the center of the meat piece towards the
411 area of crust formation. This decrease was further promoted by increasing the
412 temperature, and could have impacted the formation of HAs, especially PhIP or IQx.
413 However, the analysis of the relative effects of the different precursors of HAs, and of
414 temperature and water activity in a complex media like meat would need more analysis
415 and would benefit from a transfer-reaction modelling approach (Kondjoyan et al.,
416 2014).
417
418 In terms of practical implications, we found specific heating conditions that maintain a
419 roast beef meat aspect while dramatically reducing HAs content in the meat subjected to
420 hot air jet conditions. However, further thermal studies will be needed to mitigate HAs
421 formation while maintaining the roast beef meat aspect considering different types of
422 cooking equipments. Sensory analysis will also be needed to analyse the impact of these
423 heating conditions on savour and flavour.
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512

513 **Figure legends**

514

515 **Figure 1**: Schematic representation of the sampling procedure for the measurement of
516 crust thickness, the quantification of HAs content, and the quantification of creatine in
517 five slices of meat.

518

519 **Figure 2**: Quantification of creatine at different depths in meat (subjected to a 192 °C
520 air jet during 60 min). Average creatine value in raw meat (1.9 g/100g FDP) is plotted
521 as a solid line. Dotted lines correspond to the standard deviation. For information
522 purposes, creatine concentrations are expressed both in g / 100 g FDP and in mg/100 g
523 cooked meat to facilitate comparison with literature data.

524

525 **Figure 3**: Schematic representation of the thickness of the different areas in the crust
526 after meat cylinder was subjected to the air jet heating at 210 °C for 70 min. These
527 thickness values are issues from the work of Portanguen et al. (2014).

528

529

530

Figures

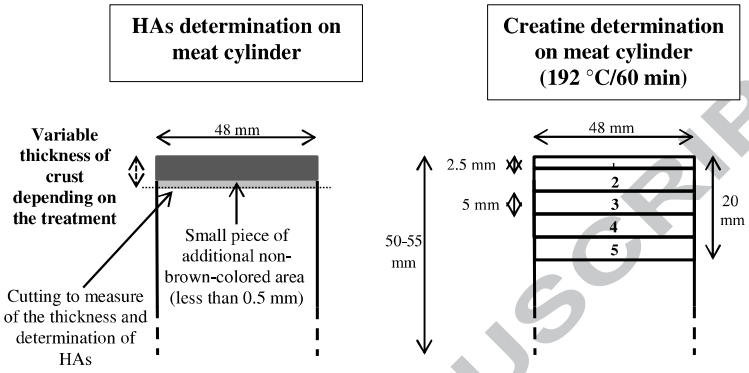

Figure 1

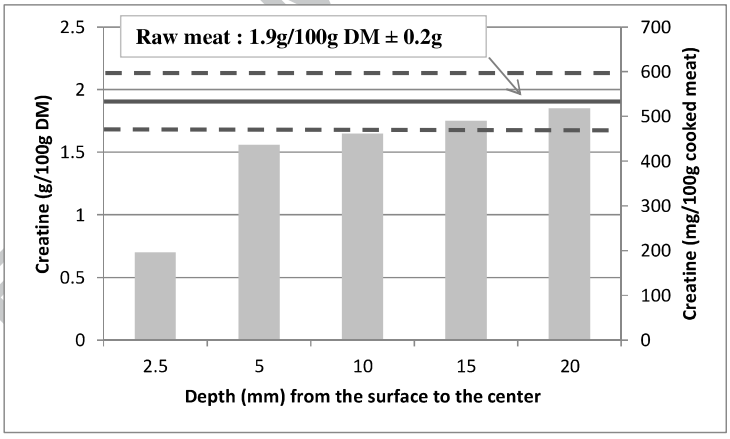

Figure 2

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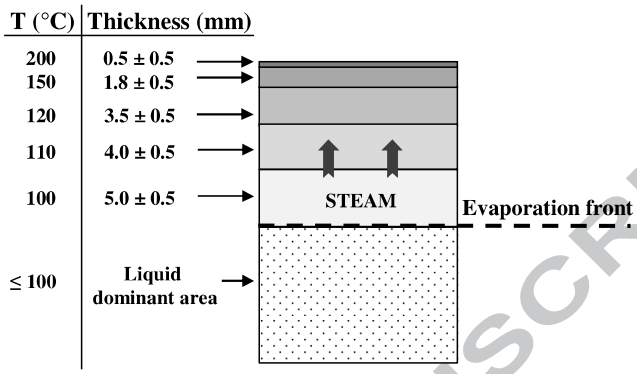

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551 **Figure 3**

552

Table legends

Table 1: Heating times and temperatures corresponding to HAs measurements.

Table 2: Creatine and creatinine levels in beef depending on cooking conditions.

Table 3: Detailed formation of HA content in the developing meat crust (variation of crust thickness) given in nanograms of compound per gram of FDP (including the crust and the piece of additional non-brown-colored area). Values highlighted in grey: no significant effect of the cooking time on HA concentration. The line 5 gives the heating condition which maintain the roasted aspect meat while reducing HAs content in the meat.
* Crust too thick, not accepted by consumers.

Tables

Table 1

T_{pipe} (°C)	T_{jet} (°C)	Cooking Time (min)
160	120 to 130	20, 40 and 90
190	150 to 160	20, 40
225	190 to 200	20, 30, 60 and 90
260	200 to 220	20, 40, 60 and 90

Table 2

Meat	Cooking method	Cooking temp. (°C)	Cooking time (min)	Creatine (mg/100g)	Creatinine (mg/100g)	Authors
Stewing-beef	Raw	/	/	364.5	7.8	Harris et al. (1997)
	Boiling	98	10-60	346.2-285.9	26.1-118.8	
Beef (*ST*)	Raw	/	/	401.0	5.8	Purchas et al. (2004)
Beef (*TB*)	Raw	/	/	329.0	5.6	Purchas et al. (2005)
Beef (*LL*)	Raw	/	/	383.5	6.1	Purchas et al. (2006)
	Grilling	71	/	310.6	43.2	
Beef (*LD*)	Raw	/	/	600.0	19.0	Polak et al. (2009)
Deep frozen beef patties (70g, 8 mm thick x 113 x 105 mm)	Raw	/	/	343.0*	9.9*	Gibis et al. (2010)
	Fried	230	2-3	226.9-178.5*	52.8-116.9*	
Beef (*LT*)	Raw	/	/	/	10.3	Kondjoyan et al. (2010b)
Beef (*SM*)	Raw	/	/	/	10.9	
Beef (LT)	Raw	/	/	470.7	/	Present study
	Baking	192	20	416.9	/	
		210	90	101.0	/	

*Levels based on dry matter.

Table 3

Lines	Roasting time (min)	Roasting Temp (°C)	Crust thickness (mm)	MeIQx (ng/g FDP)	IQx (ng/g FDP)	4,8-DiMeIQx (ng/g FDP)	PhIP (ng/g FDP)	MeIQ (ng/g FDP)	DMIP (ng/g FDP)	Glu-P-2 (ng/g FDP)
1	20	124	0.17	1.3 ±0.2	nd	nd	0.3 ±0.1	nd	nd	nd
2	40		0.19	19.4 ±0.6	1.7 ±0.2	nd	5.1 ±0.2	traces	nd	nd
3	90		0.42	41.1 ±7.0	2.3 ±0.1	2.2 ±0.1	5.8 ±0.7	traces	8.7 ±0.9	0.5
4	20	158	0.38	10.9 ±5.7	0.51	traces	3.6 ±1.4	traces	nd	nd
5	40		0.72	13.7 ±1.3	nd	traces	1.2 ±0.5	traces	2.9	trace
6	20	192	0.86	35.5 ±0.5	2.2 ±0.3	2.0 ±0.1	14.5 ±1.5	nd	6.3 ± 0.7	traces
7	30		1.42	88.5 ±8.8	5.1 ±0.6	5.2 ±0.2	21.0 ±1.6	nd	13.1 ±1.9	traces
8	90		3.85	217.0 ±1.6	9.8 ±0.3	11.9 ±0.2	98.0 ±2.2	1.1 ±0.2	41.0 ±1.5	21.6 ±0.7
9	20	210	1.94	181.1 ±2.1	10.3 ±0.3	11.4 ±0.3	95.0 ±1.7	0.5 ±0.2	27.7 ±0.4	9.4 ±0.5
10	40		2.59	220.4 ±8.6	11.7 ±1.0	14.0 ±0.5	156.9 ±4.8	1.8 ±0.2	48.8 ±2.2	24.9 ±1.4
11*	90		5.82	417.0 ±14.8	21.6 ±1.0	26.9 ±0.9	255.9 ± 10.5	2.7 ±0.2	99.7 ±6.1	48.0 ±5.9