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Formation of heterocyclic amines in slices of *Longissimus thoracis* beef muscle subjected to jets of superheated steam

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ABSTRACT

The kinetics of the formation of heterocyclic amines (HA) were measured on slices of *Longissimus thoracis* (LT) muscle subjected to impinging jets of superheated steam. Product temperature was either 170 or 200 °C and treatment duration ranged from 1 to 20 min. The concentrations of IQx, MeIQx, 4,8-DiMeIQx and PhIP followed regular kinetic patterns. HA formation increased significantly between 170 and 200 °C. The quantities of IQx and 4,8-DiMeIQx formed in LT slices were 3 to 4-fold smaller than those formed in meat juices, while quantities of MeIQx and PhIP remained comparable. A first-order kinetic model taken from the literature was adapted to describe the results taking into account product temperature variations over the course of the experiment.

1. Introduction

Heterocyclic Amines (HA) are formed in trace amounts in cooked muscles. To date, more than 20 HA have been isolated as potent mutagens. HA formation rate increases with temperature. The temperature range of 150–200 °C, which is widely used for grilling and often roasting, enhances HA formation, whereas at temperatures ranging from 225 to 250 °C the HAs begin to degrade or react with other compounds (Chiu & Chen, 2000). Moreover, water activity is always an important factor in HA formation (Borgen, Solyakov, & Skog, 2001).

It has been demonstrated that both meat and gravy are important sources of HAs (Skog, 2002). However, insufficient current knowledge on the exact quantities produced under different cooking conditions leads to epidemiological studies wrongly estimating consumer exposure levels (Aleajos, Gonzalez, & Afonso, 2008). As Aleajos et al. (2008) pointed out: one of the main problems in epidemiological studies is that many published experiments were performed under unspecified cooking conditions or using high cooking temperatures over long periods to maximise HA production. The degree of doneness (meat surface colour) often used to characterise the heat treatment (Sinha et al., 1998) of meat pieces is only a rough indicator, and is far short of sufficiently characterising cooking conditions. In fact, most of the contradictions in the literature are due to uncertainty over the time–temperature history of the surface region ("crust") where HAs are formed. Furthermore, as there is no precise definition of the "crust" region, the quantity of HAs formed therein is divided by the total mass of the meat piece, which will include a large but unknown proportion of meat treated at lower temperature and where HAs have no chance of forming.

Heat and mass transfer modelling can be employed to analyze the time–temperature history in the surface region of the meat, but transfer model-simulated predictions of HA formation require an identified reaction model (Tran, Salmon, Knize, & Covin, 2002). HA formation kinetics experiments have been run in liquid model and meat juice systems (Arvidsson, van Boekel, Skog, & Jägerstad, 1997; Arvidsson, van Boekel, Skog, Solyakov, & Jägerstad, 1999). HA formation in model systems follows a first-order reaction, thus suggesting that it could be explained by a monomolecular or bimolecular reaction where there is a large excess of one of the components (Arvidsson et al., 1997, 1999). However, Borgen et al. (2001) have shown that meat matrices can yield variable results that differ strongly from measured recordings in liquid systems. Hence, it is unclear whether liquid reaction models can be applied in more complex solid systems such as meat matrices.

Kinetics of formation of HAs have been measured by Ahn and Grün (2005) in 5 g ground beef samples containing 16% of fat and heated in glass test tubes (13-mm inner diameter and 1.0-mm wall thickness). Five to ten minutes of lag time was observed before HAs formation and after 20 min of heating HAs contents were 30–40 times smaller (expressed in ng/g product) than what was observed in liquid media. However, part of this lag time was

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probably due to thermal inertia and difference in HAs content included the effects of temperature gradients inside product. HAs formation has been measured recently in meats cooked under different domestic conditions (Ni, McNaughton, LeMaster, Sinha, & Turesky, 2008; Turesky, Taylor, Schnackenberg, Freeman, & Holland, 2005). HAs contents found in beef meat were generally much lower than those measured by Ahn and Grün (2005), but these results cannot really be interpreted kinetically due to the lack of knowledge of product surface temperature and of the unknown proportion of the meat piece subjected to low temperatures.

This study applied highly precise controlled heating conditions on 1–2 mm meat slices of lean *Longissimus thoracis* muscle subjected to a superheated steam jet. Surface temperature was continually monitored throughout the treatment. The spatial uniformity of temperature in the whole slice was analyzed using a heat transfer model. The amount of HAs formed in the meat was determined at different treatment times. Ten different HAs were studied, including polar (IQ, IQx, MeIQ, MeIQx, 4.8 DiMeIQx, PhIP) as well as non polar (TrpP1, TrpP2, AαC and MeAαC) HAs, mostly classified as possible (class 2B) or probable (class 2A) human carcinogens by the International Agency for Research on Cancer (IARC, 1987a; IARC, 1987b; IARC, 1993). Although Harman and Norharman are known to be formed during beef meat cooking, they were not studied since they can be considered as only "co-mutagenic" compounds (Totsuka, Takamura-Enya, Nishigaki, Sugimura, & Wakabayashi, 2004). The effect of time and temperature on HAs formation was analyzed, modelled and compared to literature results for liquid systems and for beef meat.

2. Materials and methods

2.1. Raw sample preparation and analysis

Longissimus thoracis and a few *semimembranosus* muscles were taken from carcasses of 18-month-old heifers immediately after slaughter. The muscles were cut in big pieces and aged for 12 days under vacuum-packed conditions. Then they were frozen and stored at −20 °C. Fat content in beef pieces was ranging between 3% and 5%. Some of the samples were used for biochemical analysis while others were kept for thermal treatments. The thawed muscles were analyzed biochemically to measure pH and determine content in creatinine, glycogen, glucose-6-P, glucose, phenylalanine, tyrosine and tryptophan. Glucose, creatinine and previous amino acids are known to be important precursors of HAs while glycogen and glucose-6-P can generate glucose during heating.

Aromatic amino acids (tryptophan, tyrosine, and phenylalanine) were determined by a second derivative spectrophotometry method (Gatellier et al., 2008). All measurements were performed in triplicate. Values were expressed as μmol/g meat.

Creatinine was determined according to Jaffé (1886) by reaction with alkaline picrate to form a coloured creatinine picrate complex. Meat extracts were prepared by homogenisation of 1 g of meat in 10 ml of 0.5 M perchloric acid with Polytron for 30 s. One millilitre of working reagent (sodium hydroxide 200 mM + disodium phosphate 12.5 mM + picric acid 8.75 mM) was added to 0.1 ml of meat extract. All chemicals were purchased from Sigma Chemical Co. Absorbance at 492 nm was measured against a water blank 30 s after mixing reagent and sample and after a 2-min incubation at 37 °C, and the absorbance increase was calculated. Creatinine in meat extract was evaluated by comparison with a standard (20 mg/ml of creatinine) treated in the same conditions. Values were expressed as μmol/g meat.

Glycogen was determined from about 1 g of muscle which was homogenised in 10 ml of 0.5 M perchloric acid. Glycogen, glucose and glucose-6-phosphate were determined on the homogenate according to Dalrymple and Hamm (1973), after hydrolysis of glycogen with amyloglucosidase. Concentrations were expressed as μmol/g fresh tissue.

The big pieces of meat which intended for heat treatment were thawed in a water bath at 15 °C, and a 5 cm-diameter cylinder was cut from the centre. This cylinder was then sliced to obtain 1–2 mm-thick disc-shaped slices.

2.2. Heat treatment and temperature measurements

Kondjoyan and Portanguen (2008) gave a detailed description of the experimental apparatus with functional analysis. Steam produced by a generator operating under 5–7 bars of pressure (steam temperature, 150–165 °C) was released into a stainless steel pipe channelling the steam to the sample. Superheated steam was obtained via electrical resistances heating the pipe. A straight pipe section was then bent to position the outlet just above the sample surface. During these experiments, superheated steam temperature at the outlet was either 180 or 500 °C, and the distance (d) between the sample surface and the pipe outlet was set at either 36 or 83 mm. The average temperature of the jet impinging the sample was either 120, 190 or 220 °C; however, this temperature did vary during the treatment. Sample and support were positioned on a sliding device mounted on a ball-bearing system. The temperature at the sample surface was measured using a digital IR pyrometer (IN 500, Impact system) over a 20 mm-diameter spot at the centre of the sample. The measuring part of the IR system was attached to the sliding device to enable continuous sample surface temperature measurements throughout the heating treatment. IR pyrometer response was calibrated using the procedure described in Kondjoyan and Portanguen (2008). The temperature of the impinging jet was measured every second using a 0.5 mm-thick calibrated type K thermocouple positioned 3.0 mm above the centre of the sample surface. Before each experiment, the apparatus was kept under the same heating conditions for at least 4 h so as to achieve steady-state conditions before the experiments began. The sample was first moved 300 mm away from the superheated steam jet, after which steam was released for a few minutes to purge the system and to limit droplet formation in the jet. The sample was then slid beneath the jet. At the end of the heat treatment, the sample surface was rapidly cooled by sliding the sample beneath a 45–55 ms^{-1} jet flow of cold air (temperature 3–5 °C) produced by a Ranque-Hilsch "vortex tube". The three positions of the sliding system, i.e., sample away from the superheated steam jet, sample impinged by jet, and sample subjected to cold air, were set perfectly using a blocking ball-bearing system. Relative humidity in the jet was directly dependent on the temperature of the superheated steam. Relative humidity was 12.8% at impinging jet temperature of 170 °C and 0.25% at jet temperature 250 °C.

Treatment durations were set according to results found by Arvidsson et al. (1999) backed up by preliminary trials on the slices.

2.3. Identification and quantification of HAs

An LC-APCI-MS/MS method previously developed for the determination of HAs in poultry extracts (Chevolleau, Touzet, Jamin, Tulliez, & Debrauwer, 2007) was adapted and optimised to quantify 10 HAs in beef meat extracts. The protocol was modified to reach a maximum recovery yield for each HA during extraction and purification steps.

One gram of lyophilised beef meat was treated with 12 ml of 1 M NaOH, during 1 h and 30 min and under stirring. The internal standard (TriMeIQx) was added at this stage at the concentration of 50 pg/μl. A liquid/liquid extraction was performed on a 13 g Extrelut (Merck) with 75 ml methylene chloride. The extract was

purified on an Oasis MCX (60 mg, 3 ml) cartridge and then evaporated to dryness. The solid residue was then redissolved in 200 μl of the HPLC mobile phase A for LC-MS/MS analysis. The recovery yields obtained with this optimised protocol were in the 20% (TrpP1, TrpP2) to 70% (MeIQx, DiMeIQx, AαC) range, with a mean variation coefficient of 5.3% (n = 3). These yields were slightly inferior to those obtained in the previous work on chicken meat (Chevolleau et al., 2007). Both ethyl acetate and methylene chloride were tested as extraction solvents and the latter was found better. Although the reverse situation was reported on meat juice systems (Borgen et al., 2001), methylene chloride is the most used extraction solvent for works developed on meat (Ahn & Grün, 2005; Chevolleau et al., 2007; Ni et al., 2008, Turesky et al., 2005).

The LC separation was performed at 25 °C on a Chromsep Pursuit C8 column (150 × 2 mm, 3 μm) (Varian) fitted with a guard column. The mobile phases consisted of A: ammonium acetate (30 mM, pH 5) and B: acetonitrile/methanol (2/1, v/v). Elution was achieved using initial conditions at 90% A, followed by a linear gradient from 10% to 15% B between 0 and 1 min, then from 15% to 30% B between 1 and 6 min, isocratic at 30% B between 6 and 12 min, linear gradient from 30% to 55% B between 12 and 15 min, isocratic at 55% B between 15 and 18 min, linear gradient linear from 55% to 70% B between 18 and 21 min, followed by isocratic conditions at 70% B between 21 and 30 min. The flow rate was 0.2 ml/min and the volume injected was 20 μl.

Mass spectrometric detection was achieved on a triple quadripole (TSQ Quantum, Thermo Fisher, Les Ulis, France) mass spectrometer using positive APCI ionisation. Mass detector conditions were: corona needle discharge current: 4 μA, nebulizer temperature: 300 °C, heated transfer capillary temperature: 300 °C, transfer capillary voltage: 35 V, tube lens offset: 104 V, sheath gas flow rate: 25 arb. units, and auxiliary gas flow rate: 5 arb. units. An internal standard (TriMeIQx, 50 pg/μl) was used for calibration at the following levels: 1, 10, 50, 100, 500, 1000 and 2000 pg/μl.

The performance of the method was determined in terms of linearity (r^2 > 0.99 between 1 and 2000 pg/μl), repeatability of retention times (mean VC ~ 0.3%, n = 5) and area ratios (mean VC ~ 2.8, n = 5), as well as limit of detection (between 0.02 and 0.1 ng/g) and limit of quantification (between 0.04 and 0.2 ng/g). There was no matrix effect (post-column addition of the 11 HA mixture) on three different muscle samples cooked for 1 min in a microwave oven. Each cooked meat sample was extracted and analysed in triplicate. HA contents were expressed as nanograms of compound per gram of freeze-dried product.

2.4. Reaction modelling

The model was a simple first-order reaction model employed by Arvidsson et al. (1999) to predict the formation of HAs in meat juice, i.e.

$$C_t = I_0(1 - \exp(-k(t - t_0))) \quad (1)$$

where C_t is the concentration at time t, t_0 the lag-time to reaction, and k the rate constant of HA formation. I_0 corresponded to the initial concentration of an unknown intermediate from which HA was formed. I_0 was also equal to HA concentration for an infinite duration C_∞ if degradation did not occur. Arvidsson et al. (1999) offset a fixed I_0 value for each temperature T. In present study, I_0 was considered to be a linear function of T, and relation (1) was discretized over time to account for temperature variation in the meat slice during heat treatment. The increase in HA concentration from C_{t-1} to C_t during a time step δt was described by:

$$C_t = (A + BT)k\exp(-k(t - t_0))\delta t + C_{t-1} \quad (2)$$

where k was assumed to be constant during the time-step and was calculated by Eyring's relation:

$$K = \frac{k_b T}{h}\exp\left(\frac{\Delta S}{R}\right)\exp\left(-\frac{\Delta H}{RT}\right) \quad (3)$$

where k_b is the Boltzmann constant (1.381×10^{-23} J K^{-1}), h is the Planck constant (6.626×10^{-34} J s), R is the molar gas constant (8.3145 J mol^{-1} K^{-1}), ΔS is activation entropy (J mol^{-1} K), and ΔH is activation enthalpy (J mol^{-1}). In some cases, HA concentrations reached a maximum concentration C_{max} at a given time t_{max} before being subject to degradation. HA degradation during a time-step δt was described by:

$$C_t = -k_2(C_{max} - C_\infty)\exp(-k_2(t - t_{max}))\delta t + C_{t-1} \quad (4)$$

where C_∞ is the HA concentration at an infinite time and k_2 is the rate constant of HA degradation. As the temperature during degradation was almost constant, k_2 was at first approximation assumed to be independent of temperature.

The temperature T values introduced into the model were taken from the calibrated IR measurements. The time-step used for discretization was the IR measurement acquisition rate, i.e. 0.2 s.

Parameters A, B, ΔS, ΔH, k_2 and C_{inf} were obtained by minimisation of the sum of squared differences (SSD) between calculated and experimental values. The fmincon function in Matlab 7.0 was used to find the constrained minimum of the SSD. The minimisation process was stopped when SSD variation during the last 50 calculation steps was less than 1% of the SSD value.

3. Results and discussion

3.1. Sample composition temperature and water activity

3.1.1. Raw sample composition

The pH of the *L. thoracis* and the *semimembranosus* muscles was 5.50 ± 0.06, thus in accordance with the pH of normal aged beef meat. Similar to meat juice, the small variation of pH observed here had no significant effect on the results and was therefore excluded as a variable for the rest of this study.

Sample content in aromatic amino acids, creatinine and saccharides is given in Table 1. The samples presented fairly constant tryptophan, phenylalanine and tyrosine contents in line with published figures for beef meat, i.e. 0.52–1.09/100, 0.53–1.18/100 and about 0.23/100 g, respectively (Subrt, Kracmar, & Divis, 2002). Creatinine remained also constant between samples and in the range cited in literature for beef muscle (Purchas, Rutherfurd, Pearce, Vather, & Wilkinson, 2004). Conversely, there were significant variations in glycogen, glucose and glucose-6-P content, which is known to vary a lot in muscle depending on physiological factors and ageing conditions. Creatinine contents in the LT and SM muscles were comparable, if expressed on a dry-mater basis, to what was measured in the meat juice used by Arvidsson et al. (1999), while amino-acids content was much more important in the muscles than in the juice. In contrast to amino-acids, the glucose content was much more important in the meat juice than in the LT and SM muscles.

3.1.2. Sample temperature and a_w during heating treatment

When the temperature of the jet impinging the sample reached about 120 °C, the temperature at the sample surface rose rapidly to a plateau and thereafter remained constant and equal to the boiling point of water throughout the experiment (Kondjoyan & Portanguen, 2008).

Fig. 1 gives the average sample-surface temperatures across the full sample set under both the highest-temperature superheated steam jet treatments. The standard deviation between measured temperatures over the course of the experiments was 5–7% of the average value of the curve. The surface temperature of the meat slice under heat treatment can never be considered constant.

Table 1
Determination of the concentrations of specific aromatic amino acids and carbohydrates in the three *Longissimus thoracis* muscles and in *semimembranosus* muscle used in the experiments. The compounds targeted are known to be important precursors of HA formation.

Muscle/animal	Phenylalanine		Tyrosine		Tryptophane		Glucose	G6P	Glycogen	Creatinine	
	g/100 g	μmol/g	g/100 g	μmol/g	g/100 g	μmol/g	μmol/g	μmol/g	μmol/g	mg/100 g	μmol/g
LT1	0.62	37.5	0.31	17.1	0.17	8.3	12.16	5.96	6.03	11.43	1.01
LT2	0.68	41.1	0.61	33.6	0.2	9.8	15.32	7.85	38.74	11.04	0.97
LT3	0.74	44.7	0.40	22.1	0.17	8.3	5.90	3.11	23.81	11.27	1.00
SM	0.58	35.1	0.42	23.2	0.15	7.3	9.01	5.49	40.90	10.32	0.91

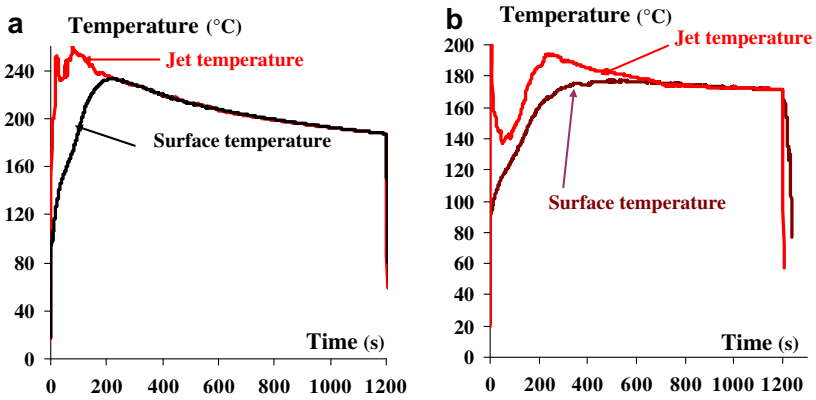

Fig. 1. Time-course of the temperature of the superheated steam jet and at the sample surface during a 20-min heat treatment: for (a, left) the "200 °C" treatment and (b, right) the 170 °C treatment. Pipe temperature is 500 °C and the distance between the pipe outlet and the sample is 36 mm at left (a) and 83 mm at right (b).

It increased sharply at the start of the treatment, then diminished slowly in parallel with jet temperature. Under the less-intense heating conditions (jet outlet-to-sample distance, d = 83 mm), the average surface temperature was 142, 158.9 and 166 °C after 300, 600 and 1200 s of treatment, respectively. Under the more intense treatment (d = 36 mm), these same time-point temperatures were 200, 208 and 202 °C, respectively. To simplify this report, these treatments will now be referred to as "170 °C treatment" and "200 °C treatment". The superheated steam jet temperature dropped faster under the 200 °C treatment than under the 170 °C treatment. The difference in surface temperatures under each experimental treatment therefore narrows over time. Thus, the between-treatment temperature difference was around 30 °C after 600 s and around 15 °C after 1200 s. This made it difficult to envisage introducing a further temperature threshold between the '170 °C' and '200 °C' treatments under this experimental design.

Calculations were run, although not detailed here, to determine the temperature difference between the upper part of the sample slice impinged by the jet, and the bottom part laid on the support. The results showed that the temperature difference between the core of the meat slice and its surface was important during the first 60 s of treatment and decreased afterwards. The average difference for temperatures greater than 150 °C, corresponding to HAs formation, was 5% of the surface temperature value (i.e. ±10 °C at 200 °C). This difference was of the same magnitude as the between-treatment temperature difference, and could therefore be introduced into the experimental error. Therefore, the calibrated surface temperature was thereafter considered as the temperature of the entire meat sample, within the bounds of experimental error.

Sample weight loss and in-sample moisture activity patterns are illustrated in Fig. 2a and b. As expected, sample weight loss increased over time whereas sample water activity decreased. The greater the temperature of the superheated steam jet the drier the jet. This resulted in greater sample weight loss and lower sample water activity. Total cumulative weight loss recorded at the end of the treatment was around 65% of the initial sample weight. Sample water activity quickly became very low. Under the 200 °C treatment, sample water activity dropped to 0.5 and then 0.2 after 8 and 10 min of treatment, respectively.

3.2. HA formation

The treatment under which sample temperature was kept constant at 98 °C was taken as a blank test. HAs were accordingly either non-detectable or detected in trace quantities only, except for MeIQx and PhIP concentrations which were at around 0.2–0.3 ng/g freeze-dried sample.

Under the 170 and 200 °C treatments, TrpP1 and TrpP2 were never detected, or were only detected in quantities which were below the quantification threshold. A weak and non-systematic formation of compounds IQ, MeIQ, AαC and MeAαC was observed. MeAαC exceeded the detection threshold in only one case (temperature of 170 °C and treatment times of over 5 min) and in very low concentrations, ranging from 0.29 to 0.49 ng/g. MeIQ was found above the quantification threshold in one single case (concentration around 4.0 ng/g) for a temperature of 200 °C and at treatment times of over 5 min. IQ was detectable in several experiments and in equivalent amounts at both 170 and 200 °C. IQ concentration increased linearly with heating time, from 0.8 ng/g after 3 min to around 3 ng/g after 20 min of treatment. AαC showed a similar pattern between 5 and 10 min of treatment, with an increase in concentration from 0.4 ng/g after 5 min to 3.0 ng/g after 10 min of treatment. After 10 min of treatment, AαC concentration decreased when the sample was at 200 °C whereas it continued to increase when the sample was at 170 °C, reaching 6.4 ng/g after 20 min of treatment. The non polar HAs: TrpP1, TrpP2, AαC and MeAαC are known to be pyrolitic mutagens only produced in important quantities when the temperature of the cooked muscle is above 300 °C

(Ahn & Grün, 2005). In this study food temperature never exceeded 240 °C which explains the small amounts of these non polar HAs found in the cooked muscles. The lesser quantity of the polar HAs: IQ and MeIQ found in this study compared to IQx and MeIQx are also in accordance with the results obtained in literature in beef meat for the greatest time–temperature conditions (Ahn & Grün, 2005; Ni et al., 2008).

The four other polar amines IQx, MeIQx, 4,8-DiMeIQx and PhIP showed concentration patterns that were regular and repeatable in relation to time and temperature. No significant increases were recorded while sample temperature stayed below 150 °C, i.e. in the first 2 min under the 200 °C treatment and in the first 4 min under the 170 °C treatment. However, the increase in HAs was only really significant after 3 and 5 min of 200 °C treatment and 170 °C treatment, respectively. Measurements of the kinetics of HA formation are given in Fig. 3; error bars indicate standard deviations on the measurements. Some of the deviations are so slight that they are hidden by the experimental target point. Standard deviations do however appear higher for the 200 °C temperature points and 7–10 min treatment times, which is explained by the fact that it

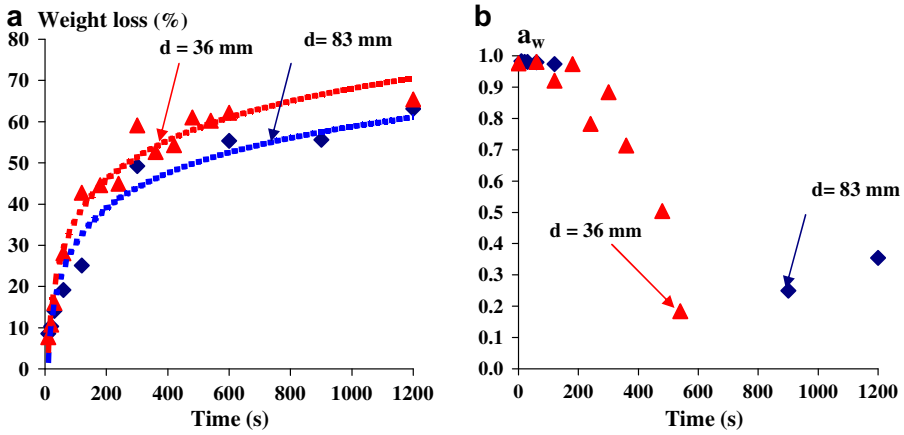

Fig. 2. Time-course of sample weight loss (a, left) and water activity (b, right) during a 20-min heat treatment "at 200 °C" (triangles, tube = 500 °C and d = 36 mm) and "at 170 °C" (diamonds, tube = 500 °C and d = 83 mm).

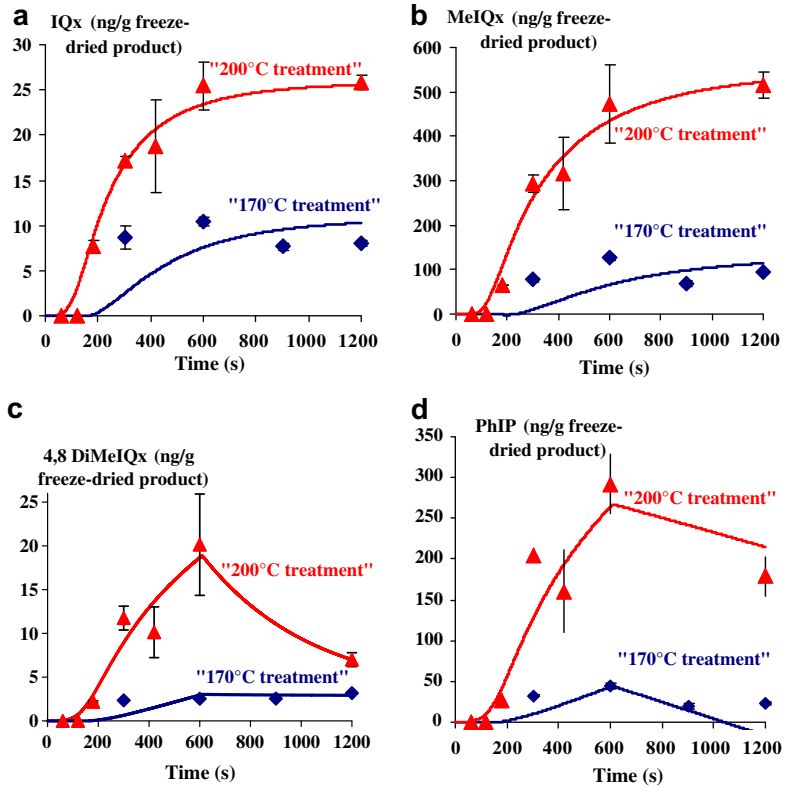

Fig. 3. Time-course of HA formation during the "170" and "200 °C" heat treatments (top left (a), IQx; top right (b), MeIQx; down left (c), 4.8 DiMeIQx; down right (d), PhIP). Error bars show standard deviations on the measurements of HA concentrations. When bars are not visible, they are just very small and hidden by the dots. Lines are the calculations derived from the kinetics model using the same parameters for both the "170" and the "200 °C" treatments (Table 3). A better description of the data (not shown) can be obtained by considering the results from the "170" and the "200 °C" treatments separately. But the more general form of the model was preferred here to be compared to data.

proved more difficult to obtain a perfectly repeatable time–temperature history with the highly superheated steam jets.

The lag times measured in this study are close to those found in the meat juice by Arvidsson et al. (1999). The close connection found between lag time and the time needed for product temperature to become greater than 150 °C (Figs. 1 and 3) confirm our assumption of an important effect of thermal inertia on lag time, especially in solids.

The results confirm the sharp increase in HA formation in meat and meat juices between 170 and 200 °C reported across the board. The quantities of IQx, MeIQx, PhIP and 4,8-DiMeIQx formed after 5 min of cooking increased 2.5-fold, 3.8-fold, 6.0-fold and 8.0-fold, respectively, when the temperature increased from 170 to 200 °C. The quantities of MeIQx and PhIP formed in the *L. thoracis* were particularly high, reaching over 250 ng/g after 10 min of treatment, IQx and MeIQx concentrations plateaued whereas 4,8-DiMeIQx and PhIP concentrations decreased. Arvidsson et al. (1997, 1999) and Chiu and Chen (2000) also reported stronger decreases in these two HAs in liquid media in the 200–225 °C temperature range and after longer periods of heating.

The amounts of HAs formed in this study are of comparable magnitude as those found in meat juice while they are more than 20 times greater than those found in ground beef by Ahn and Grün (2005). This important difference can mostly be explained by the heterogeneity of temperature which should have existed in Grün and Ahn's samples and, to a lesser extent, by a difference in fat content.

Table 2 compares HA contents in *L. thoracis* slices to the HA contents in meat juices reported by Arvidsson et al. (1999) under identical time–temperature conditions. Comparative analysis reveals that the ratio of HAs formed in solids *versus* liquids decreases as treatment time or sample temperature increases. The quantities of IQx and 4,8-DiMeIQx formed in *L. thoracis* slices were on average 3 to 4-fold lower than the quantities formed in meat juices, whereas the amounts of MeIQx and PhIP formed remained comparable between the two media. Since large quantities of PhIP are formed in 'dry cooking' systems (Borgen et al., 2001), the high amounts measured here may have been due to the low water activity during the superheated steam treatments (Fig. 2b). It has been established that more MeIQx is formed in beef and pork than in chicken, but although MeIQx formation is normally accelerated by 'liquid-based' cooking techniques, this was not the case in our study. This apparently divergent result needs to be explored by dissociating the time-course patterns of temperature change from the time-course patterns of water activity, which is extremely difficult to do in real-world conditions. Spot time-point comparisons also conceal broad disparities, even when working only with temperatures and cooking times as factors. In particular, the disparities can be heavier for longer cooking times due to the degradation of HA, as is the case for 4,8-DiMeIQx which is formed in 10-fold lower quantities in meat slices than in meat juices when the product was heated to 200 °C for 20 min. Similarly, the quantity of PhIP produced in a slice of *L. thoracis* muscle is 1.5-fold higher than in meat juice after a 5-min cooking time, but this proportion drops to 0.45 after a 20-min cooking time.

It is difficult to know what proportion of the difference in HAs content between LT muscle and juice can be explained by: (1) thermal and water activity effects, (2) difference in composition and (3) other specific "matrix" effects. A pure effect of composition could be connected to the higher glucose content found in meat juice compared to LT muscle while the difference in the content of amino acids is more difficult to interpret (Table 1). However, during our experiments a three fold multiplication of the glucose content in the raw LT muscle did not lead to significant difference in the HAs content of cooked muscles.

Table 2
Ratio of the HA content found in the heated *Longissimus thoracis* slices to the HA content found under the same time–temperature conditions by Arvidsson et al. (1999) in a meat juice system.

HAA	Temperature = 170 °C			Temperature = 200 °C		
	5 min	10 min	20 min	5 min	10 min	20 min
IQx	0.35	0.27	0.18	0.21	0.19	0.14
MeIQx	1.68	1.17	0.47	0.83	0.78	0.60
4,8-DiMeIQx	0.68	0.33	0.22	0.38	0.33	0.07
PhIP	3.77	2.26	0.59	1.55	1.14	0.45

3.3. Modelling of reaction kinetics

The model developed by Arvidsson et al. (1999) introduced a lag-time into the chemical reactions that varied according to temperature conditions. We opted not to follow this procedure here, as the time taken for the meat surface to reach 150 °C would almost certainly have been longer that the time lapse before the reactions were triggered. This temperature-based threshold was therefore set as value t_0 in Eq. (1). The limited number of hold-temperature steps considered in the present study should make it mathematically possible to describe the experimental results based on a very high number values for activation enthalpy and activation entropy. However, our experimental results still shared similar patterns with the dynamics of HA formation in liquid media. We therefore decided to boundary the ΔH and ΔS values based on the parameter values obtained in this medium as calculated by Arvidsson et al. (1999): $16{,}000\ \mathrm{J\ mol^{-1}} \leqslant \Delta H \leqslant 72{,}000\ \mathrm{J\ mol^{-1}}$, $-235\ \mathrm{J\ mol^{-1}\ K^{-1}} \leqslant \Delta S \leqslant -117\ \mathrm{J\ mol^{-1}\ K^{-1}}$ were the resulting constant k in Eq. (3) is expressed in $\mathrm{min^{-1}}$. The parameters of the solid meat matrix model were adjusted to describe the kinetics recorded for each hold-temperature threshold taken either separately or simultaneously. The constraints placed on ΔH and ΔS were not always able to lead to realistic-enough values for rate constant k. When the rate constants proved unrealistic, an additional constraint was added to keep the rate constant to below the $0.45\ \mathrm{min^{-1}}$ threshold, i.e. at a similar level to the rate constant measured for PhIP in liquid media at 225 °C ($0.419 + 0.104\ \mathrm{min^{-1}}$). Table 3 reports the parameters and residues derived through this constraint-bounded optimisation step.

The values for rate constant k, which were determined for IQx, MeIQx and 4,8-DiMeIQx by simultaneously processing the results obtained at two temperature levels and by boundarying ΔH and ΔS, were slightly higher than the rate constants obtained in liquid media. However, the rate constant determined for PhIP was not realistic, and the simulation run at 170 °C led to negative concentrations. This was very likely due to the significant difference between the PhIP values measured at the two temperature levels. Adjusting the parameters to incorporate only the values obtained at 200 °C made it possible to decrease residues for all the HA studied without straying away from the k values previously determined. However, although adjusting the parameters to incorporate only the values obtained at 170 °C did manage to generate a decrease in residues, it also led to rate constant k values way above those obtained at 200 °C, which in kinetics terms is an unrealistic pattern. Boundarying the rate constant made it possible to output realistic k values without generating an unacceptable increase in residues. Except for PhIP simulations, the simultaneous adjustment step that maintains a more general-overview model should be preferred over adjustments to temperature level, even if it outputs greater residues. This preferred model leads to a fairly good representation of the experimental results (Fig. 3). Since this study was kept limited to two hold-temperature thresholds, we cannot determine ΔH and ΔS with any greater certainty,

Table 3
Parameters of the kinetic relations (2)–(4) derived from the minimisation procedure under different constrained conditions. ΔH and ΔS are the reaction activation enthalpy (J mol^{-1}) and activation entropy (J mol^{-1} K^{-1}), respectively. k (min^{-1}) and k_2 (min^{-1}) are the rate constants of HA formation and HA degradation, respectively. SSR is the sum of the square residues between calculated and experimental results.

H.A.A.	Temperature (°C)	k constrained	a	b	ΔH	ΔS	c_{inf}	k_2	SSR		k (min^{-1})		Remark
									170 °C	200 °C	170 °C	200 °C	
IQx	170 and 200		0.42	−175.9	16000	−226.5			56	18	0.177	0.249	
	170	No	0.43	−177.4	22237	−191.3			5		2.240		Unrealistic k
		Yes	0.00	10.1	16000	−219.0			14		0.436		
	200		0.78	−348.7	28441	−200.9				9		0.229	
MeIQx	170 and 200		10.51	−4540.9	16000	−231.4			9443	6312	0.098	0.138	
	170	No	0.61	−113.0	21182	−139.8			1920		1466.000		Unrealistic k
		Yes	0.00	107.5	16000	−219.0			2101		0.436		
	200		18.56	−8443.4	22625	−219.0				4331		0.114	
PhIP	170 and 200		7483.56	−2179.4	54989	−235.2	1672.8	−0.004	2103	9690	0.000	4.43E−06	Neg. values
			5.72	−2503.6	16000	−225.2			1853	14365	0.207	0.292	
	170	No	0.49	−176.3	20304	−186.0			362		6.840		Unrealistic k
		Yes	0.00	34.6	16000	−219.0			552		0.436		
	200		7.59	−2155.0	66073	−146.8	161.2	0.1624		7191		0.011	
DiMeIQx	170 and 200		3.08	−1224.4	34885	−219	2.89	0.1383	3	23	0.003	0.005	
	170	No	0.43	−181.6	22538	−190.2			0		2.350		
		Yes	0.00	3.7	19998	−219.0			2		0.147		
	200		0.75	−338.7	16009	−235.0	6.84	0.4300		28		0.0893	

Table 4
HA concentrations (in ng/g of freeze-dried product) found after a 10-min heat treatment at 200 °C in samples of *Longissimus thoracis* (LT) and *semimembranosus* muscle (SM), respectively. Ratio of the SM to the LT HA content.

HAA	LT (ng/g)	SM (ng/g)	SM/LT
IQx	25.5	16.2	0.6
MeIQx	473.1	280.8	0.6
4,8-DiMeIQx	20.1	12.3	0.6
PhIP	292.3	105.8	0.4

and consequently we cannot further discuss the underlying reaction mechanisms (unimolecular or bi-molecular reactions, etc.)

3.4. Generalizability to other muscles

We ran a series of prospective experiments where the *L. thoracis* was replaced by *semimembranosus* beef muscle and where the meat was heated to 200 °C for 5 min (Table 4). The *semimembranosus* muscle produced 40% lower quantities of IQx, MeIQx and 4,8-DiMeIQx than *L. thoracis*, and 60% less PhIP. However, pH and precursor composition (except for glycogen content, which *a priori* does not enter directly into the reactions) were similar between the two modelled muscles when raw (Table 1). Fat content is known to affect HA formation (Hwang & Nagdi, 2002), but since both *L. thoracis* and *semimembranosus* are lean muscles taken from animals slaughtered at the same fat score, fat content cannot explain the muscle differences in HA concentrations. Therefore, this difference can only be explained by: (1) variations in reaction precursors or reaction intermediates not assayed in this study, and/or (2) resulting differences in moisture and/or precursor transfers at tissue scale. This significant between-muscle difference reported here needs to be further confirmed through cooking experiments carried out under other time–temperature conditions.

4. Conclusion

Accurate controlled heating conditions were applied on 1–2 mm meat slices of lean *L. thoracis* muscle subjected to superheated steam jets. The four polar amines IQx, MeIQx, 4,8-DiMeIQx and PhIP, which are thermic mutagens, were found in greater amount than the apolar pyrolitic mutagens, which is consistent with the level of temperature reached during this study. These thermic mutagens are generally considered as more harmful than the pyrolitic mutagens. IQx, MeIQx, 4,8-DiMeIQx and PhIP followed regular and repeatable kinetic patterns in relation to time and temperature. Consistently with other reports, we were unable to detect significant increases in HA formation at temperatures of 150 °C or less, whereas there was a sharp increase in HA formation at temperatures close to the 200 °C mark.

The analysis of heat transfer and the measure of product surface temperature proved the importance of thermal inertia on the lag time of HAs formation especially in solid foods. The amounts of HAs formed in this study were comparable in magnitude to those found in meat juice while they are more than 20 times greater than those found in literature for ground beef. This difference in HAs contents found in solids highlighted the necessity to take into account the temperature gradients to interpret the kinetic study conducted in solid foods.

The quantities of IQx and 4,8-DiMeIQx formed in *L. thoracis* slices were on average 3 to 4-fold lower than the quantities formed in meat juices, whereas the amounts of MeIQx and PhIP formed remained comparable between the two media. These averaged evaluations actually camouflage broad disparities according to temperature and cooking time. The key findings in terms of human health risks relate to the 200 °C threshold, where the model predicts significantly lower quantities of HAs being produced than in the 'meat juices' model, except for PhIP which is formed at peak rates under shorter cooking times (5 min) such as those used for grilling meat.

The structure of the first-order kinetics model developed for liquid media can be adapted to describe the results achieved on *L. thoracis* muscle, provided that continuously-monitored in-sample temperature variations are factored into the model. However, since the present study factored in relatively few temperature levels, the parameters cannot be determined with the same level of precisions as for liquid media. Indeed, it remains difficult to control temperature drift between superheated steam jets using the current experimental set-up. This technical difficulty can be overcome in the future, for example by replacing steam jets with heated-air jets, which are easier to work with.

Comparative analysis was run in one experiment condition by replacing *L. thoracis* muscle with *semimembranosus* muscle. The

semimembranosus produced an average 40% less HA than the *L. thoracis*, and 60% less PhIP. These findings, which cannot be explained by composition-related differences, remain to be confirmed for other time–temperature conditions.

The differences in HA concentrations measured between *L. thoracis* and meat juice and between *L. thoracis* and *semimembranosus* muscle highlight the importance of working with solid meat matrices to complete the assessment of the consumer risks involved in exposure to HA.

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