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EVALUATION OF HETEROCYCLIC AMINES IN AN AQUEOUS AND MEAT MATRIX BASED MODEL SYSTEM

Alexander Schoch, Monika Gibis, and Albert Fischer

Department of Meat Technology, Insitute of Food Technology, University of Hohenheim, 70599-Stuttgart, Germany

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Background:

The quality of meat can be considered under aspects of nutritition, flavour and toxicology. Toxicological characteristics of cooked meat could be due to the content of Nitrosamines, Polycyclic Aromatic Hydrocarbons (PAHs) or Heterocyclic Amines (HAs). Heterocyclic Amines have been shown to be potent mutagens and carcinogens and consequently the International Agency for Research on Cancer (IARC) has recommended the reduction of human exposure to HAs [1]. One way to follow this is eating less thermally processed meat or to inhibit the formation of HAs during heating.

To study the influence of different additives on HA formation, model systems containing the HA precursors creatin/ine, amino acids and sugars are useful tools. This is due to the fact that HAs isolated from such model systems are identical with mutagens isolated from cooked meat. Up to now, three main types of model systems have been described in literature: dry-heating of the precursors [2], reflux boiling in diethyleneglycol/-water mixtures [3], or heating the aqueous solution in closed metal vessels [4].

Objectives:

The aim of our work was to characterise the physical and chemical parameters of HA formation in an easy-to-handle model system. This should allow the carrying out of a large series of aqueous and meat matrix based experiments under identical conditions, without the use of expensive equipment or time and work consuming clean up steps.

Methods:

Heating Conditions: All experiments were carried out in an autoclav model FVS/3, Fedegari (Italy) with a maximum temperature of 140°C, maximum pressure 2.5 bar, total volume 140 L. Temperature measurement was carried out using a Pt 100 in the autoclav which allows the temperature to be controled by 0.1°C. Furthermore, we measured for every step of temperature (105°C; 110°C; 115°C; 120°C; 125°C; 130°C; 135°C) the pressure in the autoclav: 0.35 bar; 0.58 bar; 0.85 bar; 1.15 bar; 1.55 bar; 1.93 bar and 2.4 bar, respectively.

Time and temperature experiments: All chemicals and solvents are HPLC grade or p.a.14 mmol creatinine, 14 mmol glycine and 7 mmol glucose were dissolved in 50 mL Milli-Q water and put in a 100 mL glass bottle which was closed with an aluminium film. After heat processing, the samples were refilled up to 50 mL with Milli-Q water to avoid inaccurancy due to unequal loss of water. Temperature dependent experiments were carried out at the temperatures mentioned above. The heating time for each step was 60 min. Time dependent experiments were carried out at the highest heating step (135 °C) with a hold-time from 10 to 70 min, in 10 min steps.

Glucose dependent experiments: 14 mmol creatinine and 14 mmol glycine were mixed with 1.4 mmol; 4.2 mmol; 7 mmol; 10.5 mmol; 14 mmol; 21 mmol and 28 mmol glucose, respectively, dissolved in Milli-Q waterand thermally processed for 60 min at 135 °C.

Meat matrix based experiments: 70 g of very finely chopped beef was heated at 135 °C for 2 ½ h. Meat analysis data: 73.6 % water, 20.2 % protein, 5.2 % fat, 1.1 % ash (methods see [5]). The content of creatine (0.466 %) and creatinine (< 0.006 %) was analysed with an enzymatic test kit [6]. After the heat processing step, Milli-Q water was added to the samples up to 70 g to avoid inaccurancy due to unequal loss of water. For the extraction procedure, the samples were mixed with an Ultra Turrax (Fa. Janke und Kunkel, Staufen, Germany) and were stored at -20 °C until analysed.

Sample clean up and HPLC analysis was performed by using the method of Gross and Grüter [7] with some individual modifications.

Results and discussions:

Figure 1 shows the influence of the heating temperature on HA formation and indicates that IQx and 7,8-DiMeIQx is formed from temperatures of 110 °C and up, while MeIQx is not formed at temperatures below 125 °C. This indicates that HAs could be formed at moderate temperatures between 110 and 135 °C. This result is in agreement with work from Jägerstad [3] and Negeshi [8] who could detect 4.4 nmol MeIQx and 1.1 nmol 7,8-DiMeIQx per mmol creatinine by reflux boiling the same precursors at 128 °C for 2 h. Furthermore a study of Lee [9] indicates that a temperature of only 102 °C could be enough for HA formation in boiled pork juice when heated for 12 h. In contrast to our findings, the model system of Jackson and Hargraves [4] show no formation of HAs at temperatures below 150 °C.

Our data in figure 2 0show that the time dependent formation of IQx, MeIQx, and 7,8-DiMeIQx indicates a more linear relationship. A study of Knize et al [10] also shows that the mutagenicity of fried hamburgers increases in a linear manner with the heating time, which is mainly contributed to the content of HAs.

When glucose is added in higher amounts than half of creatinine in aqueous model systems [11] or is used excessively as a additive in hamburgers [12], a decrease of HAs could be noticed. This inhibiting potency of a higher content of glucose could also be observed by our data shown in figure 3.

In figure 4, typical UV/HPLC chromatograms of the heating experiments are presented: number 1 - standard mix solution (at bottom), number 2 - meat matrix based model system (middle) and number 3 - aqueous creatinine/glycine/glucose model system, heated 60 min at 130 °C (top).

When the meat matrix was heated at 135 °C for 2 ½ h, MeIQx could be quantified at 2.04 ng/g (s.d. 0.36 ng/g), IQx at 0.35 ng/g (s.d. 0.17 ng/g) and trace amounts of 4,8-DiMeIQx. Furthermore, the β-carbolines Norharman and Harman could be identified.
Studies of Isaacs and Coulson [13] and from Tamaoka [14] show that high pressure between 50 and 800 bar could slow down the Maillard reaction. With our autoclav heating device we reach, at 135°C, a maximum pressure of about 2.4 bar. In spite of this, we conclude that the influence of pressure is negligible for this model system.

Figure 1 :

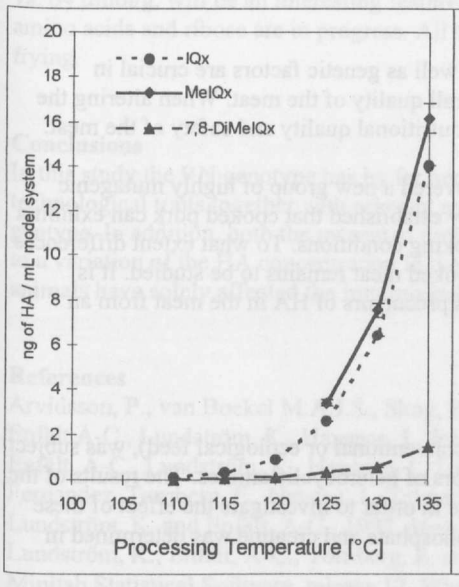


Figure 2 :

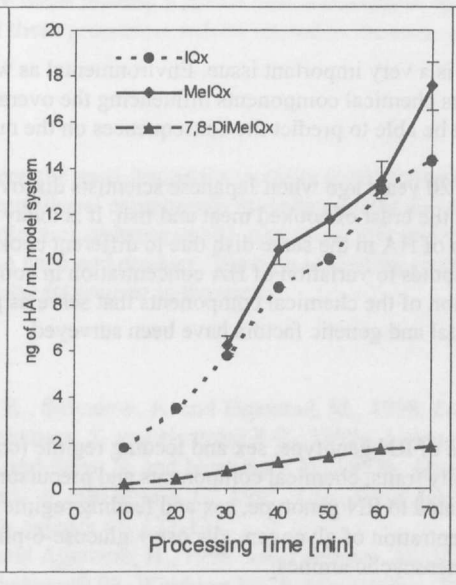
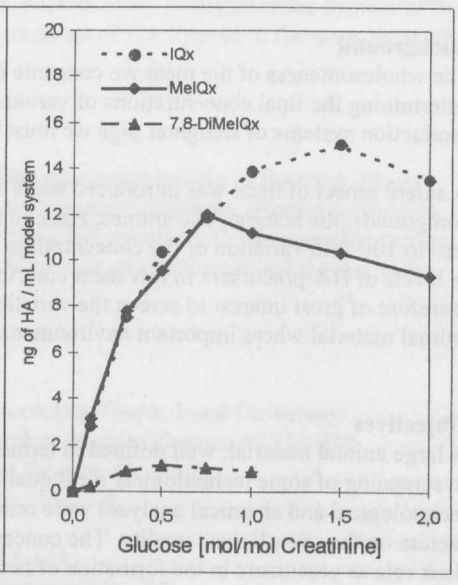


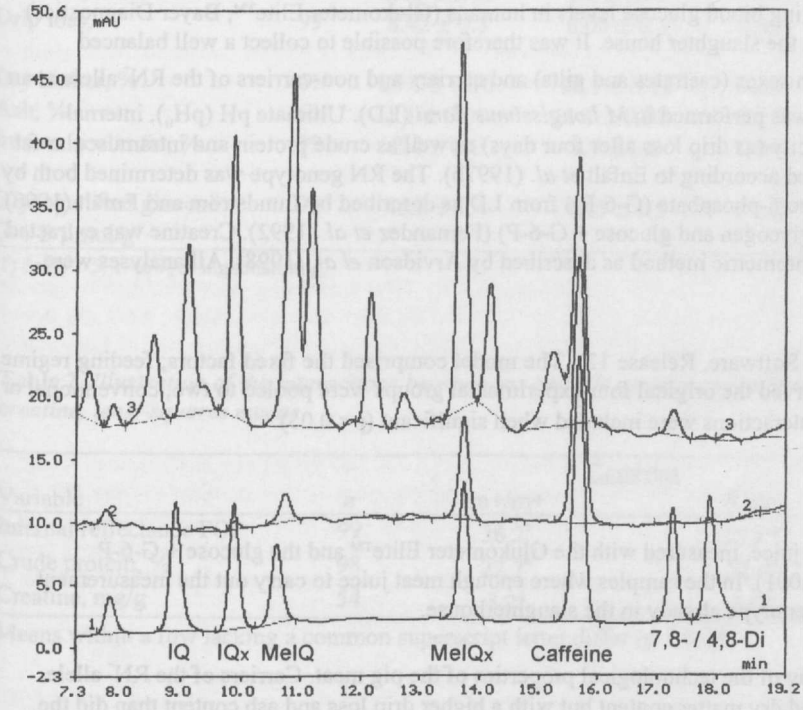
Figure 3 :



Conclusions:

The aim of this study was to characterise the physical and chemical parameters of a new model system for the formation of HAs. Compared with other heating methods, the use of a laboratory autoclav has different advantages: no Diethyleneglycol has to be used and no expensive closed metal tubes are necessary. Furthermore, this heating device allows the processing of large scale experiments under the same conditions with reproducible and exact control of pressure and temperature.

Figure 4:



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