PS6.04 Prediction of Heterocyclic Amines formation during cooking in non-marinated beef meat. 223.00

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Abstract—Heterocylcic Amines (HAs) are carcinogenic compounds found at the surface of roasted and grilled meat. Estimation of consumer exposure to HAs remains under debate in epidemiological studies. The formation of heterocyclic amines was modeled in slices of longissimus thoracis (LT) and semimembranosus (SM) beef muscle subjected to jets of superheated steam and of hot air at temperatures ranging from 170°C to 250°C and treatment times ranging from 1 to 20 minutes. The quantities of IQx and 4,8-DiMeIQx formed in LT slices were smaller than those given in literature for meat juices, while MeIQx and PhIP formation is related to the water content or activity in the meat sample. A first-order kinetics model taken from the literature was adapted to describe the results obtained on meat.

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I. 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°C-200°C, which is widely used for grilling and oven roasting, enhances HA formation, whereas at temperatures ranging from 225°C to 250°C the HAs begin to degrade or react with other compounds [1]. It has been demonstrated that both meat and gravy are important sources of HAs [2]. However, insufficient current knowledge on the exact quantities produced under different cooking conditions leads to epidemiological studies wrongly estimating consumer exposure levels [3]. Highly precise controlled heating conditions were applied on 1-2 mm meat slices of lean Longissimus thoracis (LT) and semimembranosus (SM) muscles subjected to a superheated steam [4] or hot air jet. The amount of HAs formed in the meat was determined at different treatment times. This paper reports on the main results and conclusions obtained on the polar HAs (IQ, IQx, MeIQ, MeIQx, 4.8 DiMeIQx, PhIP) which are the most important ones found in meat cooked in ovens or using electrical grills.

II. MATERIALS AND METHODS

Longissimus thoracis and semimembranosus 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. Some of the samples were used for biochemical analysis while others were kept for thermal treatments. Biochemical analyses of thawed muscles consisted in measuring pH and determining the muscle content of creatinine, glucose 6P, glucose, phenylalanine, tyrosine and tryptophan, which are known to be important precursors of HA formation. We also determined glycogen content, as thermal treatment favors glycogen hydrolysis and thus the release of glucose [4]. The heating apparatus was designed to treat 1-2 mm meat slices using either superheated steam jets [5] or hot air jets. The temperature of the impinging superheated jet was either 190°C or 220°C while the temperature of the hot air jet was close to either 170°C, 210°C, 250°C, 300°C or 400°C. The exact temperature value of the impinging jet was measured second-by-second using a 0.5 mm-thick calibrated type K thermocouple positioned 3.0 mm above the middle of the sample surface. The temperature at the sample surface was measured using a calibrated digital IR pyrometer [5]. During the experiment, the sample was slid beneath the hot jet. At the end of the heat treatment, the sample

surface was rapidly cooled by sliding the sample beneath a jet flow of cold air. HA determinations were carried out by LC-APCI-MS/MS according to a method previously developed for chicken meat and adapted for beef meat [4]. Briefly, one gram of lyophilized beef meat was first treated with NaOH 1M.

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), and evaporation to dryness, the resulting residue was re-dissolved in 200 µl of the starting LC mobile phase. Separation was performed using a Chromsep Pursuit C8 column (Varian, 150 x 2 mm, 3 µm) fitted with a guard column, and a gradient elution of mobile phases A: AcONH4 (30 mM, pH5) and B: ACN/MeOH (2/1). Then, 20 µL of the final extract was injected, and separation was performed at 25°C at a flow rate of 0.2ml/min. Mass spectrometric detection was carried out on a TSQ Quantum (Thermo Electron) triple quadrupole mass spectrometer using positive APCI ionization. The reaction model was a first-order reaction model adapted from a literature used to predict HA formation in meat juices [6].

III. RESULTS AND DISCUSSION

The four polar amines IQx, MeIQx, 4,8-DiMeIQx and PhIP showed concentration patterns that were regular and repeatable in relation to time and temperature. An example of the kinetics recorded in LT muscle for IQx under superheated-steam jet and under hot-air jet is reported in Figure 1. The error bars indicating standard deviations of the measurements are given for temperatures $\leq$ 250°C. These deviations were significantly weaker for hot air than during the experiments run with superheated steam, due to the better control over the temperatures in the air jets, and were mostly blanketed by the experimental point spread. In contrast, standard deviation became very high (approaching 40% of measured concentration) at air jet temperatures of 300°C and 400°C due to the violent structural breakdown of the product making it difficult to control the experiment. The kinetics measurements on LT were consistent with literature results. HA formation, which was weak at 170°C, increased significantly between 210°C and 250°C (except for 4,8-DiMeIQx which increased between 170°C and 210°C). This increase appeared to level off at jet temperatures in the 250°C to 400°C range. However, for the short-burst treatment times considered, average sample temperatures remained similar, regardless of jet temperature. There was an intense level of HA formation during the first 600s of treatment. After the 600s thresholds, HA can concentration either remained constant or dropped significantly depending on the type of heterocyclic amine and on the treatment condition.

The amounts of IQx and MeIQx were substantially higher under superheated steam treatment than with the dry hot air jets, but the concentrations obtained were of a similar order of magnitude. PhIP and 4,8-DiMeIQx, however, showed radically different patterns. Given that the samples shared exactly the same pH and composition in HA precursors and were subjected to comparable temperature kinetics, the differences observed were very likely due to differences in water content or in water activity. It is also possible to compare the values recorded here with meat slices [4] with the values reported in juice system [6]. As soon as temperature is close to 200°C, HA formation in slices of LT muscle subjected to jet treatments was considerably lower than in experiments carried out on meat juice, except for PhIP.

The concentrations of 4,8-DiMeIQx, IQx and MeIQx formed in slices of LT subjected to hot air jet represented only 3-4%, 11-13% and 26%-50%, respectively, of the concentrations measured in meat juice. This effect was radically different for PhIP, which formed at 137% to 550% higher concentrations than predicted by liquid media model. HA formation in LT meat can be described using the same basic first-order kinetic model and similar activation enthalpy and activation entropy values to those used in a meat juice system [6] but some of the parameters of the model have to be transformed to become temperature dependent.

This means that the reaction mechanisms in a meat matrix and in meat juice should be similar, with a bimolecular rate-limiting step (one of the two reactants being in large excess) for DiMeIQx, 4,8-DiMeIQx and IQx and a monomolecular reaction for PhIP [7]. However, the enthalpy and entropy values of the different HAs are different and are not ordered in the same way as in meat juice, except with PhIP which has greater entropy values than all the other HAs in both meat matrix and meat juice. HA formation in

semimembranosus muscle was also measured at two temperature levels, i.e. 210°C and 250°C. Figure 2 gives SM-LT ratios between HA quantities measured in semimembranosus (SM) and longissimus thoracis (LT) muscle at a hot air jet temperature of 210°C. SM-LT ratio decreases through to the end of treatment. For PhIP and IQx, the ratio of quantities formed was greater than 1 after a 300s treatment but dropped to markedly below 1.0 as soon as treatment time crossed the 600s threshold. MeIQx and DiMeIQx showed a smoother decrease. After 1200 s at 210°C, SM-LT ratio was 0.24 for PhIP and between 0.40 and 0.55 for the three other HAs. Similar behavior was observed at 250°C.

The LT and SM muscles were both lean and of comparable fat content. The two raw muscles also had similar pH and precursor composition, except for phenylalanine and for glycogen, which has to be hydrolyzed before affecting HA formation. Difference between the results could be explained by difference in water migration. The meat juices even appeared to visibly release differently from the two different muscles during cooking. Either way, the strong differences between quantities of 4,8-DiMeIQx and PhIP formed when the same type of muscle (LT) is subjected to either hot-air jets or superheated steam confirms the key role of water activity in HA formation in meat slices (previously observed in model systems). It is therefore crucial to factor water activity into future kinetic models of HA formation.

IV. CONCLUSION

Results prove that in the meat matrixes 4,8-DiMeIQx, IQx and MeIQx form in smaller quantities in meat than in meat juice. Calculating the concentrations of these HAs based on the kinetic model meat juice system would result in a 2 to 10-fold overestimation of the actual degree of consumer exposure when consumers do not ingest the juice. In contrast the PhIP concentrations measured in the LT muscle were 1.4 to 5.5-fold higher than those predicted by the 'juice model'. Predicting HAs formation in ordinary cooking conditions requires to combine kinetic models with transfer models that give reliable value of the temperature and water content "in the crust" which develops at the meat surface during its roasting and grilling. Such a work is in progress in the Prosafebeef project

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REFERENCES

[1] Chiu, C.P., & Chen, B.H. (2000). Stability of heterocyclic amines during heating. Food Chemistry, 68(3), 267-272.

[2] Skog, K. (2002). Problems associated with the determination of heterocyclic amines in cooked foods and human exposure. Food Chemistry Toxicology, 40, 1197-1203.

[3] Aleajos, M.S., Gonzalez, V., & Afonso, A.M. (2008). Exposure to heterocyclic aromatic amines from the consumption of cooked red meat and its effect on human cancer risk: a review. Additive and Contaminants, 25(1), 2-24.

[4] Kondjoyan, A., Chevolleau, S., Grève, E., Gatellier, P., Santé-Lhoutellier, V., Bruel, S., Touzet, C., Portanguen, S., & Debrauwer, L. (2009). Formation of Heterocyclic Amines in slices of Longissimus thoracis beef muscle subjected to jets of superheated steam (accepted for publication in Food chemistry DOI: 10.1016/j.foodchem.2009.02.081).

[5] Kondjoyan, A., & Portanguen, S. (2008). Prediction of surface and "under surface" temperatures on poultry muscles and poultry skins subjected to jets of superheated steam, Food Research International, 41(1), 16-30.

[6] Arvidsson, P., van Boekel, M.A.J.S., Skog, K., Solyakov, A., & Jägerstad, M. (1999). Formation of heterocyclic amines in a meat juice model system. Journal of Food Science, 64(2), 216-221.

[7]Jägerstad, M., Skog, K., Arvidsson, P., & Solyakov, M (1998). Chemistry formation and occurrence of genotoxic heterocyclic amines identified in model systems and cooked foods. Z. Lebensm. Unters Forsch. A., 207, 419-427.