COLOUR PHOTOGRAPHS TO ESTIMATE HETEROCYCLIC AMINE INTAKE FROM PORK OF DIFFERENT RN GENOTYPES

Viktoria Olsson, Kerstin Skog*), Kerstin Lundström and Margaretha Jägerstad

Department of Food Science, Swedish University of Agricultural Sciences, Box 7051, SE-750 07 Uppsala, Sweden
*) Department of Food technology, Engineering and Nutrition, Lund University, Box 124, SE-221 00 Lund, Sweden

Background

The Maillard reaction is important to obtain an appetizing surface browning of meat during frying and roasting; however, it also induces the formation of several carcinogenic/mutagenic heterocyclic amines (HCAs) (Jägerstad *et al.*, 1983). Cooked meat and fish are important sources of exposure to HCAs, as are pan residues when used to prepare gravy. The most abundant HCAs in cooked foods include 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) and 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) (Skog *et al.*, 1998). The formation of HCAs depends on cooking conditions, especially temperature and time, the concentration of precursors, such as free amino acids, creatine/ine and sugar in the food as well as the occurrence of certain modulators, e.g. pro/antioxidants (Skog *et al.*, 1998).

Although eight HCAs have been judged as probable human carcinogens and one as a possible carcinogen (group 2B and 2A respectively, according to (IARC, 1993)) it is difficult to evaluate their impact in the aethiology of human cancer. The most serious drawbacks in epidemiological studies include difficulties in estimating the exposure, for example the intake of HCAs in a population because of large ranges in the reported level of HCAs in cooked food, differences in estimated consumption (e.g. portion size, frequency), as well as difficulties in taking various cooking practices into consideration (Augustsson & Steineck, 2000). One common way to indirectly assess HCA intake is through food frequency questionnaires, sometimes complemented with colour photographs so that the degree of doneness can be estimated (Augustsson *et al.*, 1999; Sinha *et al.*, 1999). It has been suggested that visual aids, such as photographs, will improve exposure assessment in epidemiological investigations of HCA intake (Keating *et al.*, 2000). However, the relationship between cooking conditions (temperature and time), degree of surface browning and concentration of HCAs as judged from colour photographs has limitations. We have shown that pig meat of different genotypes (the dominant RN^- mutation) through a marked variation in glycogen levels, may give rise not only to varying concentrations of HCAs but also degree of browning when fried under identical conditions (Olsson *et al.*, 2002). In that study, a subjective scoring of surface browning correlated negatively with the level of HCA. Thus, the degree of surface browning may be a poor indicator of HCA content, and meat with similar surface browning could contribute very differently to the daily intake of HCAs. Because the colour of the crust is an important factor for judging doneness of the domestically cocked pig meat, we wanted to investigate how these findings affected the estimated intake of HCAs from pork chops.

Pig meat is the most popular type of meat in Sweden and the estimated frequency of carriers of the RN^- allele may be as high as 60-70% in the Swedish slaughter pig population. The *RN* mutation affects several of the precursors for the formation of HCAs, and due to hyperaccumulation of glycogen in glycolytic muscles, meat from carriers of the RN^- allele exhibits markedly higher glycogen levels than the normal meat of non-carriers (Lundström *et al.*, 1996). Further, we have found that the level of creatine and some free amino acids in the meat are affected by the *RN* genotype of the pigs (Olsson *et al.*, 2002).

Objectives

The overall aim of the present study was to contribute to an improvement of HCA exposure assessment. Pork chops from different *RN* genotypes were fried at three different temperatures, photographed, whereupon crust colour was recorded and the crusts analysed for HCAs. The results were combined with a simple questionnaire to study dietary practice and preferences, and a monthly intake of mutagenic HCAs through fried pork chops was estimated (Olsson *et al.*, 2004).

Materials and methods

Two *M. longissimus dorsi* (LD) muscles of pig, weighing approximately 2.5 kg each, were selected from the commercial cutting line at the slaughterhouse in Uppsala, Sweden. Genotyping (carrier (*RN⁻/rn+)* or non-carrier (*rn+/rn+)* of the *RN⁻* allele) was performed following the procedure reported by Milan *et al.* (2000). The contents of free amino acids (FAA), dipeptides, residual glycogen, creatine and creatinine in the meat were analysed as described before (Olsson *et al.*, 2002). The *RN⁻/rn+* and *rn+/rn+* samples were vacuum-packed, stored at +4ºC for 4 days and thereafter used in the frying experiment.

The deboned meat was fried as intact 2-cm-thick chops. Before frying, the edges of the chops were slightly cut to avoid an uneven curving during frying and the meat was tempered until the mean internal temperature was 18.7 ± 1ºC. Three *RN⁻/rn+* chops at a time, followed by three *rn+/rn+* chops were fried at low (160ºC), medium (180ºC) and high (200ºC) temperatures in a cast iron pan on an ordinary household stove with a ceramic top. The chops were fried in 10 g of a commercial vegetable frying fat for 3 min per side and then the chops were left to cool to room temperature, weighed and photographed. Cooking loss was calculated as the percentage change in chop weight before and after frying.

The concentration of HCAs in the crust was analysed using a Blue chitin solid-phase extraction method. In short, the freeze-dried crust was homogenised in NaOH, mixed with diatomaceous earth and packed into a cartridge. The HCAs were extracted with ethyl acetate. The eluate was evaporated to dryness, dissolved in NaOH and applied on a Blue Chitin column. The HCAs were eluted with MeOH/ NH4OH (9:1 v/v), evaporated to dryness, dissolved in MeOH, and then analysed using LC/MS (LCQDECA ion-trap mass spectrometer, with Xcalibur software, Thermo Finnigan, San José, CA, USA), using the electrospray as ion source (Bång *et al.*, 2002). The recoveries ranged between 30-80% depending of type of HCA and recovery corrections were made before presenting the results.

In the questionnaire, the questions were aimed to provide data on consumption habits and household cooking practices for pig meat with special emphasis on the loin (pork chops), *M. longissimus dorsi* (LD). For general questions on the consumption of pig meat the respondents were asked only to consider lean meat and not mixed or further processed products such as cooked ham, sausages or bacon. Six colour photographs showing the surface colour and the interior colour of fried pork chops were presented to the respondents who were asked to indicate which of the photographs corresponded best to their normal frying practices. The six photographs represented *RN⁻/rn+* and *rn+/rn+* meat, fried at three different temperatures described previously. The questionnaire was distributed to a total of 151 people (60 men and 91 women) in Uppsala, Sweden, at three different occasions during the summer of 2002. For the analysis of the questionnaire, the respondents were divided into four age groups: 1 = 15-20, 2 = 21-40, 3 = 41-60, and 4 = 61 years and upwards.

Results and discussion

Three initial pan temperatures, 160, 180 or 200º C were chosen to mimic various domestic frying practices. After frying pork chops from one carrier and one non-carrier of the *RN⁻* allele at these different temperatures, we found the mutagenic HCAs, MeIQx, PhIP and IQx, in low concentrations in the crust. The level of mutagenic HCAs was almost ten times higher in *rn+/rn+* meat fried at 200ºC compared to the *RN⁻/rn+* chops (0.49 vs. 4.13 ng/g cooked meat). IQx was detected only in the *RN⁻/rn+* crust whereas the comutagens, harman and norharman, were detected in the crust of the *rn+/rn+* chops but not in the *RN⁻/rn+* meat. The *rn+/rn+* meat, fried at an initial pan temperature of 200ºC gave more than 20 times the amount of mutagenic HCAs compared to that fried at 160ºC, 4.13 compared to 0.18 ng/g cooked meat. PhIP was the main HCA formed. In a study by Augustsson *et al.* (1997) a 20-fold increase of PhIP levels was typically seen when increasing the frying temperature for several meat dishes from 175ºC to 225º C, and a general temperature dependency of the formation of HCAs has been repeatedly reported (e.g. Skog *et al.*, 1995).

Based on colour photographs, the respondents reported that they preferred fried chops with medium crust colour, rejecting the very pale crust of the non-carrier of the *RN⁻* allele fried at low temperatures as well as the dark brown of the carrier fried at medium/high temperature. Most respondents chose fried chops from the non-carrier, which, based on information given in a questionnaire, would result in an markedly higher average contribution to the monthly HCA intake of 359 ± 402 ng (mean ± SD) compared to 35 ± 60

ng/month for consumers who preferred the *RN⁻/rn+* chops. Overall, the total monthly intake of mutagenic HCAs derived from consuming pork chops was moderate, on average 256 ng, ranging from 0 to 1982 ng/month (Table 1). A mean intake of 256 ng/month would mean a limited daily contribution of about 9 ng from fried pork chops, well in agreement with an earlier Swedish study which estimated a daily contribution of 10.1 ng/day from fried pork chops and gravy (Voskuil *et al.*, 1999). This intake rate should be related to the assessed total daily intake of 200 and 120 ng HCAs for Swedish men and women, respectively (Augustsson *et al.*, 1997). Besides the Swedish study, data on the specific contribution of pork to the intake of HCAs are sparse. According to estimates by Layton *et al.* (1995) the intake of HCAs originating from pork would be 51 ng/day for a man or woman weighing 70 kg. The corresponding figures for beef and chicken would be 542 and 173 ng/day, respectively. The estimated daily intake in this study was however high, 1820 ng/day compared to 160 ng/day in another Swedish study (Augustsson *et al.*, 1997).

Conclusions

To estimate total HCA intake, epidemiologists try to identify markers for the factors cooking time and temperature. Doneness of meat and crust browning have been put forward as a reasonable indirect measurement of mutagenic activity (Steineck *et al.*, 1993; Sinha *et al.*, 1999). However, our data show that care should be taken when using colour photographs to determine cooking preferences for assessment of HCA intake because background factors, such as the occurrence of the *RN⁻* allele in pigs, may influence the results. The *RN* genotype of pigs affects both colour formation and HCA content and thereby makes crust colour inappropriate for estimation of HCA content in cooked pig meat carrying the *RN⁻* allele.

The study involves respondents from a small regional area in Sweden and a relatively homogenous population group. Thus, it is not appropriate to extrapolate its findings to general exposure of HCAs in the Swedish society. However, it does contribute to better knowledge about the level and formation of HCA in domestically prepared pork chops as well as understanding of the complexity of using different exposure indicators, such as the degree of surface browning, in the assessment of HCA intake through diet. The study shows that the raw material composition is important and that small adjustments in the domestic cooking practices may help reduce the formation and intake of HCAs.

References

Augustsson, K., Skog, K., Jägerstad, M., Dickman, P. W. & Steineck, G. 1999. Dietary heterocyclic amines and cancer of the colon, rectum, bladder and kidney: a population-based study. *Lancet* 353, 703-707.

Augustsson, K., Skog, K., Jägerstad, M. & Steineck, G. 1997. Assessment of the human exposure to heterocyclic amines. *Carcinogenesis* 18, 1931-1935.

Augustsson, K. & Steineck, G. 2000. Cancer risk based on epidemiological studies. In M. Nagao and T. Sugimura, *Food borne carcinogens, heterocyclic amines.* Wiley. Chichester. pp.

Bång, J., Nukaya, H. & Skog, K. 2002. Blue Chitin columns for the extraction of heterocyclic amines from cooked meat. *Journal of Chromatography A* 977, 97-105.

IARC. 1993. Monographs on the Evaluation of Carcinogenic Risk to Humans. *International Agency for Research on Cancer*, Vol. 56

Jägerstad, M., Laser Reuterswärd, A., Öste, R., Dahlqvist, A., Olsson, K., Grivas, S. & Nyhammar, T. 1983. Creatine and Maillard reaction products as precursors of mutagenic compounds formed in fried beef. In G. R. a. F. Waller, M.S., *The Maillard reaction in foods and nutrition.* American chemical society. Washington DC. 507-519 pp.

Keating, G. A., Sinha, R., Layton, D., Salmon, C. P., Knize, M. G., Bogen, K. T., Lynch, C. F. & Alavanja, M. 2000. Comparison of heterocyclic amine levels in home-cooked meats with exposure indicators (United States). *Cancer Causes & Control* 11, 731-739.

Layton, D. W., Bogen, K. T., Knize, M. G., Hatch, F. T., Johnson, V. M. & Felton, J. S. 1995. Cancer Risk of Heterocyclic Amines in Cooked Foods - an Analysis and Implications for Research. *Carcinogenesis* 16, 39-52.

Lundström, K., Andersson, A. & Hansson, I. 1996. Effect of RN gene on technological and sensory meat quality in crossbred pigs with Hampshire as terminal sire. *Meat Science* 42, 145-153.

Olsson, V., Skog, K., Lundström, K. & Jägerstad, M. 2004. Colour photographs for estimation of heterocyclic amine intake from fried pork chops of different RN genotypes indicate large variations. *Food Quality and Preference* In press,

Olsson, V., Solyakov, A., Skog, K., Lundström, K. & Jägerstad, M. 2002. Natural variations of precursors in pig meat affect the yield of heterocyclic amines - Effects of RN genotype, feeding regime, and sex. *Journal of Agricultural and Food Chemistry* 50, 2962-2969.

Sinha, R., Chow, W. H., Kulldorff, M., Denobile, J., Butler, J., Garcia-Closas, M., Weil, R., Hoover, R. N. & Rothman, N. 1999. Well-done, Grilled Red Meat Increases the Risk of Colorectal Adenomas. *Cancer Res* 59, 4320-4324.

Skog, K., Johansson, M. & Jägerstad, M. 1998. Carcinogenic heterocyclic amines in model systems and cooked foods: A review on formation, occurrence and intake. *Food and Chemical Toxicology* 36, 879-896.

Skog, K., Steineck, G., Augustsson, K. & Jägerstad, M. 1995. Effect of cooking temperature on the formation of heterocyclic amines in fried meat products and pan residues. *Carcinogenesis* 16, 861-867.

Voskuil, D. W., Augustsson, K., Dickman, P. W., van't Veer, P. & Steineck, G. 1999. Assessing the Human Intake of Heterocyclic Amines: Limited Loss of Information Using Reduced Sets of Questions. *Cancer Epidemiol Biomarkers Prev* 8, 809-814.

Table 1. *Estimated monthly intake (ng) of mutagenic HCAs from domestically cooked pork chops, based on information on how often pork chops (loin) are fried, serving size and the calculated content of HCAs for the cooked chop in the particular photograph chosen (means ± SD)*

	IQx	MeIQx	PhIP	Total mutagenic HCAs
Men, n = 58	6 ± 20	50 ± 56	189 ± 252	244 ± 305
	max 137	max 206	max 1033	max 1188
Women, n = 90	1 ± 8	52 ± 78	211 ± 327	264 ± 401
	max 80	max 413	max 1570	max 1982
Tot. population, n=148	3 ± 14	51 ± 70	202 ± 299	256 ± 365
	max 137	max 413	max 1570	max 1982