JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Natural Variations of Precursors in Pig Meat Affect the Yield of Heterocyclic Amines − Effects of RN Genotype, Feeding Regime, and Sex

VIKTORIA OLSSON,*,† ALEXEY SOLYAKOV,‡ KERSTIN SKOG,‡ KERSTIN LUNDSTRÖM,† AND MARGARETHA JÄGERSTAD†

Department of Food Science, Swedish University of Agricultural Sciences, Box 7051, SE-750 07 Uppsala, Sweden, and Department of Applied Nutrition and Food Chemistry, Center for Chemistry and Chemical Engineering, Lund University, Box 124, SE-221 00 Lund, Sweden

Pig meat shows natural variations in the concentrations of precursors of heterocyclic amines (HCAs), which may affect formation of HCAs in cooked pig meat. To study this, 26 pigs with an inherent genetic variation (carriers and noncarriers of the RN$^-$ allele) were subjected to different feeding regimes (conventional feed compared with feed composed according to organic standards). In addition, the effect of sex (castrated males or females) was considered when assessing chemical and technological meat quality parameters. Concentrations of precursors of HCAs, i.e., creatine, residual glycogen, dipeptides, and free amino acids, were analyzed in the raw meat, and the levels of some HCAs (4,8-DiMeIQx, MeIQx, PhIP, harman, and norharman) were then determined in fried meat patties prepared from these pigs. The RN genotype most affected technological meat quality parameters and the level of precursors of HCAs, especially the level of residual glycogen, where carriers of the RN$^-$ allele showed levels four times as high as those of noncarriers (75.3 ± 2.6 compared with 17.2 ± 2.4 μmol/g meat, least-squares means ± SE). The increased level of residual glycogen resulted in about 50% lower amounts of total mutagenic HCAs in cooked meat compared with cooked meat from normal pigs. Fried meat from carriers of the RN$^-$ allele obtained darker crust color than meat from noncarriers. Feeding regime and sex did not significantly affect the chemical composition of the meat or the formation of HCAs.

KEYWORDS: Pig meat quality; RN$^-$ allele; heterocyclic amines; PhIP; mutagens; precursors

INTRODUCTION

Heterocyclic amines (HCAs) can be formed at ng/g levels during the cooking of meat and fish. Currently, about 20 carcinogenic/mutagenic HCAs have been identified. Long-term studies of animals have shown that HCAs are carcinogenic at multiple sites (*1*−*5*) and DNA-adduct formation in human tissues has also been documented (*6*). Among the most abundant HCAs in cooked foods are 2-amino-1-methyl-6-phenylimidazo-[4,5-*b*]pyridine (PhIP) and 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) (*7*). Several HCAs, e.g. the IQ and IQx compounds, are formed from naturally occurring precursors in the meat: sugars, creatin(in)e, free amino acids, and some dipeptides via the Maillard reaction (*8*). The formation of HCAs depends on the cooking conditions (especially temperature and time), the concentration of modulators (e.g., pro/antioxidants), and the concentrations of the precursors already mentioned (*7*). The endogenous concentrations of the precursors in muscles vary between animal species. Therefore, meat from different species, cooked in the same way, exhibits large variations in mutagenic activity (*9*). In addition, meat from the same species and muscle type may give rise to varying amounts of HCAs (*7*, *10*). The level of PhIP, one of the most common HCAs produced in cooked pig meat (pork chops), ranges from not detectable to 4.8 ng/g; and MeIQx, from not detectable to 2.6 ng/g (*7*).

More knowledge on the natural variation of HCA precursors for each meat-producing species is needed, and systematic studies in this field are currently lacking.

The chemical composition of pig meat may be affected by genetic and environmental factors such as the occurrence of major genes or choice of feed for the animals. Over the years, a particular major gene, the dominant, so-called RN$^-$ allele, has been proven to significantly influence production and meat quality traits in the Swedish slaughter pig population, where 40−50% of the animals are RN$^-$ carriers (RN$^-$/rn+) (*11*). The dominant allele occurs in Hampshire and Hampshire crosses (*12*−*14*) causing significantly higher glycogen content in glycolytic muscles than in other pig breeds and noncarriers of the RN$^-$ allele (rn+/rn+) of the same breed (*15*−*17*). The increased glycogen concentration leads to an increased level of

* To whom correspondence should be addressed (fax +46-(0)18-673080; phone +46-(0)18-671226; e-mail viktoria.olsson@lmv.slu.se).
† Swedish University of Agricultural Sciences.
‡ Lund University.

10.1021/jf011239h CCC: $22.00
Published on Web 04/11/2002

residual glycogen, i.e., glycogen, glucose, and glucose-6-phosphate in the meat post mortem (*18*). Another important consequence of the RN^- allele is low ultimate pH of the meat, which, in combination with increased glycogen and decreased protein content, results in reduced water holding capacity in both fresh and cooked meat (*18*). The RN^- allele also positively affects meat quality: cooked meat from carriers of the RN^- allele has in sensory tests been rated as being more juicy and acidulous and as having a more intense meat taste than cooked meat from noncarriers (*18*−*20*).

In the development of more sustainable food production methods, alternatives to the conventional agrosystems are evaluated. Today, the most well-defined alternative system to produce pig meat in Sweden is the KRAV (translated as the control association for organic food production) certified production of organic meat. KRAV is a member organization of the International Federation of Organic Agriculture Movement (IFOAM) and works out standards and monitoring programs for organic food producers in Sweden. Pigs reared according to the standards of KRAV should, among other things, be able to express more of their natural behavior and have free access to roughage. Furthermore, their feed should be produced and composed according to special regulations, which means that, ideally, most of the feed should be produced on the farm where the animals are reared. The addition of synthetic amino acids to optimize the nutritional value of the feed, is not allowed in KRAV certified pig feed (*21*).

This study's main aim was to analyze the concentrations of HCAs in fried patties of pig meat, originating from the same muscle but from different animals, and to examine to what extent possible variations were associated with different meat composition. Another aim was to elucidate which components in pig meat are the most important for the formation of HCAs. Technological meat quality and chemical properties of meat from 26 pigs were investigated. The animals were either carriers or noncarriers of the RN^- allele and had been fed conventional feed or organic feed composed according to the KRAV regulations.

MATERIALS AND METHODS

Animal Material. The animal material was a subsample from a larger study designed to monitor the effect of organic feed on production and carcass traits as well as the economic return of the production system. The original animal material consisted of 240 crossbred ((Landrace × Yorkshire) × Hampshire) pigs bought from commercial herds in western Sweden. The animals were kept indoors in conventional pens. Of the 240 animals, 120 were fed a conventional feed mixture, and the other 120 animals were fed organic feed. A subsample ($n = 99$) was chosen for technological meat quality assessment, and the selection was further reduced to comprise 26 pigs (13 castrated males and 13 females, see below for selection procedure) for the analysis of chemical composition and formation of HCAs. The pigs were sent to slaughter when they reached a live weight of 107 kg. The animals were transported 30 min to a commercial abattoir, the lairage time was approximately 2 h, and stunning was carried out using CO_2.

Technological Meat Quality Assessment. Carcasses intended for meat quality assessment were transported to the Swedish Meats slaughter plant in Uppsala, Sweden, and cut the second day after slaughter. All measurements and analyses were carried out in *M. longissimus dorsi* (LD). At cutting, ultimate pH (pH_u) and drip loss were determined as previously reported by Enfält et al. (*22*). Samples of LD, weighing approximately 300 g, intended for analysis of the water, ash, intramuscular fat (IMF), and crude protein contents of the meat were cut 20 cm anterior of the last rib, frozen, and stored at −20 °C until analysis according to Enfält et al. (*22*, *23*).

Selection of Samples Representing Carriers and Noncarriers of the RN^- Allele. Initially, samples from 99 animals were classified as either carriers or noncarriers of the RN^-allele, based on two phenotypic traits: (a) measurement of the concentration of glucose + glucose-6-phosphate in the meat juice as described by Lundström and Enfält (*24*); and (b) enzymatic determination of the concentration of residual glycogen (denotes glycogen, glucose, and glucose-6-phoshate) after hydrolysis of glycogen using amyloglucosidase in muscle tissue homogenized in cold perchloric acid (*25*). Both methods gave rise to a bimodal distribution, and the RN phenotyping was done on the basis of the valley point of method (b): animals with a residual glycogen concentration ≥ 35 μmol/g meat were classified as carriers of the RN^- gene. First, animals were selected for a more thorough analysis of meat composition, precursors, and level of HCAs using principal component analysis (PCA), to ensure that the two different feeding regimes, carriers and noncarriers of the RN^- allele, and animals from both sexes were represented in a balanced mode. Second, animals with high or low residual glycogen concentrations within genotype were included to build in some variation in the model. The meat of the 26 animals chosen was finally genotyped (rn+/rn+ or RN^-/rn+) according to the procedure reported by Milan et al. (*26*).

Precursors of HCAs in the Raw Meat. From the 26 animals, samples intended for identification and quantification of free amino acids (≈400 g) and for cooking experiments (≈800 g) were taken from behind the last rib, vacuum packed, aged for 4 days at 4 °C, frozen, and stored at −20 °C. Residual glycogen was analyzed as described above. Creatine and creatinine were quantified enzymatically by a Boehringer-Mannheim end-point method applied for food samples (*27*). The mean concentration of creatinine was low, <1 μmol/g meat, and therefore only the level of creatine is reported.

The content of free amino acids (FAA) and dipeptides in the meat was analyzed using a Biotronik LC-5001 analyzer at the Department of Biochemistry, Uppsala University, Uppsala, Sweden. The analysis included aspartic acid, threonine, serine, asparagine, glutamic acid, glutamine, proline, glycine, alanine, citrulline, valine, methionine, isoleucine, leucine, tyrosine, β-alanine, phenylalanine, ornithine, lysine, arginine, and histidine, as well as taurine, urea, ammonia, and anserine (dipeptide of β-alanine and histidine). Norleucine was used as internal standard. The levels of trypthophan were lower than 0.02 μmol/g meat, which was below the level of determination. A smaller sample was run on an Alpha-Plus analyzer to determine the level of the dipeptide carnosine (β-alanyl-L-histidine).

Cooking Experiments. The meat was thawed until semi-soft and minced twice through a powered mincing machine (Electrolux Assistent, Sweden), using the final plate with holes 4 mm in diameter. The minced meat was formed into patties using Petri dishes. The patties weighed 70 g each and had a diameter of 8.5 cm and a height of 1.5 cm. The patties were fried for 3 min per side on a temperature-controlled frying device set at 200 °C. Patties (2 or 3) originating from the same animal ($n = 26$) were fried simultaneously in 10 g of commercial Swedish frying fat (Milda margarin) for each patty. The final internal temperature of the patties was measured using thermocouples. The temperature of the surface of the frying device was recorded once before the patties were applied and three times during frying using a temperature probe. After the patties had cooled, they were weighed, and the cooking loss was calculated.

Color Measurements/Estimations of Surface Browning of the Cooked Meat Crust. Lightness (L*), yellowness (b*), and redness (a*) of the crusts were recorded using a Minolta Chroma Meter CR-300 with a D_{56} light source calibrated against a white tile. These values were supplemented by a subjective color score (1 to 5; where 1 was a very light crust and 5 was a very dark crust).

Analysis of HCAs. As a first concentration step (*28*), the crust, i.e., the brown surface, of the fried patties was removed using a scalpel. From 100 g of raw meat around 16 g of crust was obtained. The crust was then stored at −20 °C until extraction. From a 4-g sample of crust pooled from fried patties from the same animal, HCAs were extracted and purified according to the solid-phase extraction method of Gross and Grüter (*29*) with some modifications (*30*). HCAs were separated using reversed-phase HPLC (*31*). The column (ODS 80 TosoHaas, 250 × 4.6 mm, 5 μ i.d.) was eluted with acetonitrile and 0.01 M triethylamine (pH adjusted to 3.6 with acetic acid). Chromatograms and spectra were obtained using a photodiode array UV detector (Varian

Table 1. Effect of RN Genotype, Feeding Regime, and Sex on the Concentration (ng/g cooked meat) of Heterocyclic Amines (least-squares means ± standard errors)[a]

	RN genotype			feed			sex		
	rn+/rn+	RN$^-$/rn+	*p* value	conventional	organic	*p* value	castrated male	female	*p* value
MeIQx	1.9 ± 0.2	1.5 ± 0.3	0.273	1.6 ± 0.3	1.8 ± 0.2	0.530	1.5 ± 0.3	1.9 ± 0.2	0.247
	(*n* = 14)	(*n* = 12)		(*n* = 12)	(*n* = 14)		(*n* = 13)	(*n* = 13)	
4,8-DiMeIQx	0.4 ± 0.1	0.2 ± 0.1	0.063	0.3 ± 0.1	0.3 ± 0.1	0.695	0.3 ± 0.1	0.3 ± 0.1	0.479
	(*n* = 14)	(*n* = 12)		(*n* = 12)	(*n* = 14)		(*n* = 13)	(*n* = 13)	
PhIP	**1.9 ± 0.3**	**0.2 ± 0.3**	**0.001**	1.0 ± 0.3	1.1 ± 0.3	0.873	0.8 ± 0.3	1.4 ± 0.	0.157
	(*n* = 14)	(*n* = 11)		(*n* = 11)	(*n* = 14)		(*n* = 12)	(*n* = 13)	
sum mutagenic HCAs	**4.2 ± 0.5**	**2.0 ± 0.5**	**0.006**	3.0 ± 0.5	3.2 ± 0.5	0.775	2.6 ± 0.5	3.6 ± 0.5	0.167
	(*n* = 14)	(*n* = 11)		(*n* = 11)	(*n* = 14)		(*n* = 12)	(*n* = 13)	
Harman	0.7 ± 0.5	1.6 ± 0.5	0.217	0.4 ± 0.6	1.9 ± 0.5	0.053	0.9 ± 0.7	1.3 ± 0.5	0.659
	(*n* = 12)	(*n* = 11)		(*n* = 10)	(*n* = 13)		(*n* = 9)	(*n* = 14)	
Norharman	1.9 ± 0.6	3.4 ± 0.6	0.094	1.8 ± 0.7	3.6 ± 0.6	0.059	2.9 ± 0.7	2.5 ± 0.5	0.650
	(*n* = 11)	(*n*=11)		(*n* = 9)	(*n* = 13)		(*n* = 8)	(*n* = 14)	

[a] Bold font denotes a significant ($p < 0.05$) effect of the fixed factors RN genotype, feeding regime, or sex.

Table 2. Effect of RN Genotype, Feeding Regime, and Sex on the Concentration (μmol/g raw meat) of Some Precursors of Heterocyclic Amines (least-squares means ± standard errors)[a]

	RN genotype			feed			sex		
	rn+/rn+ (*n* = 14)	RN$^-$/rn+ (*n* = 12)	*p* value	conventional (*n* = 12)	organic (*n* = 14)	*p* value	castrated male (*n* = 13)	female (*n* = 13)	*p* value
creatine	**36.4± 0.8**	**39.7 ± 0.8**	**0.010**	38.4 ± 0.8	37.8 ± 0.8	0.582	36.9 ± 0.8	39.2 ± 0.8	0.060
residual glycogen	**17.2 ± 2.4**	**75.3 ± 2.6**	**0.001**	45.6 ± 2.6	47.0 ± 2.4	0.691	44.9 ± 2.5	47.6 ± 2.5	0.444
glycine	**1.14 ± 0.05**	**1.36 ± 0.06**	**0.006**	1.18 ± 0.06	1.32 ± 0.05	0.077	1.32 ± 0.05	1.18 ± 0.05	0.078
ornithine	**0.19 ± 0.01**	**0.11 ± 0.02**	**0.001**	**0.12 ± 0.02**	**0.18 ± 0.02**	**0.007**	0.17 ± 0.02	0.13 ± 0.02	0.057
citrulline	0.05 ± 0.01	0.05 ± 0.01	0.636	**0.04 ± 0.01**	**0.06 ± 0.01**	**0.017**	0.05 ± 0.01	0.05 ± 0.01	0.483
β-alanine	0.36 ± 0.03	0.28 ± 0.03	0.100	**0.41 ± 0.03**	**0.24 ± 0.03**	**0.001**	0.33 ± 0.03	0.32 ± 0.03	0.947
sum FAA	10.1 ± 0.6	10.7 ± 0.6	0.521	10.6 ± 0.6	10.3 ± 0.6	0.799	10.8 ± 0.6	10.1 ± 0.6	0.405
taurine	1.95 ± 0.08	1.70 ± 0.08	*	1.77 ± 0.08	1.88 ± 0.08	*	1.81 ± 0.08	1.84 ± 0.08	0.734
ammonia	7.00 ± 0.10	6.91 ± 0.10	*	6.81 ± 0.10	7.10 ± 0.10	0.050	6.93 ± 0.10	6.98 ± 0.10	*
anserine	**0.82 ± 0.03**	**0.69 ± 0.03**	**0.007**	0.78 ± 0.03	0.72 ± 0.03	0.145	0.73 ± 0.03	0.78 ± 0.03	0.213
carnosine	**29.5 ± 0.75**	**27.2± 0.81**	**0.046**	29.4 ± 0.81	27.3 ± 0.5	0.061	28.3 ± 0.80	28.4 ± 0.80	0.889
sum dipeptides	**30.3 ± 0.8**	**27.9 ± 0.8**	**0.040**	30.2 ± 0.8	28.0 ± 0.8	0.059	29.0 ± 0.8	29.2 ± 0.8	0.055

[a] Bold font denotes a significant ($p < 0.05$) effect of the fixed factors RN genotype, feeding regime, or sex. * Indicates significant ($p < 0.05$) interaction between the fixed factors.

9065, Polychrome). HCAs were identified and quantified using retention times and the spectra from reference samples of known concentrations, run under the same conditions. The following HCAs were quantified: MeIQx, PhIP, 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (4,8-DiMeIQx), 9H-pyrido[3,4-*b*]indole (norharman), and 1-methyl-9H-pyrido[3,4-*b*]indole (harman), all obtained from Toronto Research Chemicals (Toronto, Canada). In a few cases, a UV-spectrum corresponding to the substance of interest could be detected but the amount was too small to allow proper integration. These peaks were assigned a peak area, known to be below the determination limit, to perform a statistical analysis of variance. All samples were analyzed in duplicates, and 18 of the 26 samples were run as triplicates, with one of the triplicates spiked with a known amount of reference compounds to correct for incomplete extraction recovery.

Statistical Analysis. Data were analyzed using Minitab (*32*) running Pearson's correlations and the GLM procedure. The statistical model included feeding (conventional feed or feed composed according to KRAV's organic standards), RN genotype (rn+/rn+ or RN$^-$/rn+), and sex (female or castrated male) as fixed factors. Two-way interactions between these fixed factors were included when significant ($p < 0.05$). Principal component analysis (PCA) was carried out using Unscrambler (*33*).

RESULTS

Heterocyclic Amines. All 26 samples of fried pig meat contained HCAs. The concentrations of individual and total mutagenic HCAs ranged from not detectable to 8.9 ng/g cooked meat. The amounts of MeIQx, 4,8-DiMeIQx, PhIP, harman, and norharman varied among the samples and are summarized in **Table 1**. The amount of PhIP was significantly lower in fried meat from carriers of the RN$^-$ allele ($p = 0.001$). The total amount of mutagenic HCAs was also significantly lower in fried meat from carriers of the RN$^-$ allele ($p = 0.006$). Feed or sex did not give rise to any significant differences between the amount of HCAs.

Precursors in the Raw Meat. Table 2 shows the concentrations of creatine, residual glycogen, and some free amino acids that were in some way affected by the fixed factors of RN genotype, feeding regime, and sex. The levels of creatine ranged 1.5-fold, from 29.1 to 44.2 μmol/g meat. Meat from carriers of the RN allele had significantly increased levels of creatine ($p = 0.010$) compared to meat from noncarriers, but no significant differences were obtained for feed or sex. The RN$^-$ allele was also correlated with significantly increased levels of residual glycogen ($p < 0.001$), but again, no significant differences were obtained for feed or sex. The distribution of residual glycogen in this material comprising 26 animals is shown in **Figure 3**. The ranges in residual glycogen varied 13-fold, between 7 and 91 μmol/g meat. The mean residual glycogen level in meat from noncarriers of the RN$^-$ allele was 17.2 ± 2.4 μmol/g meat ww compared with 75.3 ± 2.6 μmol/g meat wet weight in meat from carriers (least-squares means ± standard errors, **Table 2**).

A few of the concentrations of individual free amino acids and dipeptides (anserine and carnosine), were affected by RN genotype or feeding regime. The RN$^-$ allele affected the levels

Table 3. Color and Cooking Loss of the Fried Pig Meat Patties as Influenced by RN Genotype (least-squares means ± standard errors)[a]

	RN genotype		
	rn+/rn+	RN⁻/rn+	*p* value
L*	45.3 ± 1.9 (*n* = 12)	42.8 ± 1.8 (*n* = 10)	0.338
a*	**12.3 ± 0.4** (*n* = 12)	**10.6 ± 0.5** (*n* = 10)	**0.018**
b*	17.2 ± 1.0 (*n* = 12)	12.8 ± 1.0 (*n* = 10)	*
subjective color score	**2.1 ± 0.2** (*n* = 12)	**4.2 ± 0.2** (*n* = 10)	**0.001**
cooking loss, %	**33 ± 0.7** (*n* = 14)	**36 ± 0.7** (*n* =11)	**0.004**

[a] Rows in bold font denote a significant ($p < 0.05$) difference between animals of different RN genotypes. *Indicates significant ($p < 0.05$) interaction between the fixed factors.

of glycine, ornithine, and the dipeptides anserine and carnosine, whereas feeding strategy affected ornithine, citrulline, and β-alanine (**Table 2**). Neither RN genotype, feeding regime, nor sex affected the total sum of free amino acids. The levels of taurine and ammonia were subject to significant interactions between the factor pairs of feeding regime and RN genotype, and sex and RN genotype, respectively. The concentrations of the other free amino acids determined were not affected by any of the factors considered (data not shown). The concentration of individual free amino acids was low, in the ranges of 0.02−2.5 μmol/g meat, where (besides tryptophan, which occurred in concentrations below the limit of determination), citrulline had the lowest values and alanine had the highest. When the concentrations of all free amino acids were summarized, the lowest level of total free amino acids was 8.0 μmol/g meat in meat of one animal, compared with a highest level of 16.7 μmol/g in another single animal. The dipeptides, anserine and carnosine, also varied about 2-fold in the ranges of 0.5−1.1 and 20.3−35.2 μmol/g meat, respectively. This shows that rather dramatic differences in the levels of precursors of HCAs occur between individual animals within the same species and muscle.

Relationships Between Precursors and HCAs. The correlation between residual glycogen and PhIP in all samples ($n = 26$) was negative (−0.58; $p = 0.003$). In the subgroup with high residual glycogen values, representing the carriers of the RN⁻ allele, these variables correlated negatively (−0.67; $p = 0.033$) but in the group with low residual glycogen values, the concentrations of residual glycogen and PhIP did not correlate. Also, the concentrations of residual glycogen and the levels of MeIQx or 4,8-DiMeIQx formed in the cooked meat did not correlate. In addition, we found no significant correlations between the level of creatine in the raw meat and the HCAs analyzed in the cooked pig meat. The formation of the comutagens, harman and norharman, in the fried pig meat samples was not affected by the factors RN genotype and sex, but concentrations tended to be higher in the meat of organically fed pigs (**Table 1**). None of the variables measured were correlated to the level of harman. For the meat originating from carriers of the RN⁻ allele, urea and ornithine correlated positively with the level of norharman (0.67 and 0.65; $p \leq 0.042$), whereas phenylalanine correlated negatively (−0.70; $p = 0.024$).

Color Formation and Cooking Loss. Measurements using the Minolta chroma meter showed that crust of meat from noncarriers of the RN⁻ allele had significantly higher a* values, i.e., the crust had a more saturated red color (**Table 3**). The b* (yellowness) values were affected by an interaction between the fixed factors feeding regime and sex, whereas the L* (lightness) value was not affected by any of the factors studied. L*, a*, and b* values were not correlated with the residual glycogen content of the raw meat. By subjective assessment of the degree of surface browning, fried meat samples from RN⁻ carriers were scored as significantly darker (4.2 compared with 2.1; $p < 0.001$) than those of noncarriers. The subjective color scores were highly correlated (0.86; $p < 0.001$) to the residual glycogen content of the raw meat. The yield of PhIP and the subjective color score correlated negatively (−0.58; $p = 0.003$) but the other HCAs and the crust color did not correlate.

The temperature on the surface of the frying device was monitored at four times during each cooking session. When the meat patties were placed on the surface the temperature fell slightly from 205 °C to 195 °C, but gradually rose back to 205 °C during the frying. Mean and maximum temperatures of the surface of the frying device did not influence the color formation (data not shown).

The mean cooking loss, calculated as percentage of weight loss during frying, amounted to 34.4 ± 2.4% (mean ± SD) in this study. The cooking loss was significantly larger for the fried meat patties made of meat from RN⁻ carriers ($p = 0.004$; **Table 3**).

Technological Meat Quality Parameters. In this study, the RN genotype considerably affected the technological meat quality parameters measured; i.e., pigs that were carriers of the RN⁻ allele produced meat with significantly higher water and ash content, increased drip loss, and lower ultimate pH and crude protein content than meat from noncarriers (**Table 4**). These results agree with those from another study (*22*) and are in line with the standard of attainment of the effect of the RN⁻ allele on these specific traits. The strategy of feeding (conventional or organic feed) and the sex of the animals did not affect the technological meat quality traits considered in this study (data not shown).

Table 4. Effect of RN Genotype on Technological Meat Quality Parameters (least-squares means ± standard errors)[a]

		RN genotype		
	n	rn+/rn+	RN⁻/rn+	*p* value
water, %	26	**74.5 ± 0.2**	**75.3 ± 0.2**	**0.003**
crude protein, %	26	**22.8 ± 0.3**	**21.7 ± 0.3**	**0.006**
intramuscular fat (IMF), %	26	2.4 ± 0.2	2.4 ± 0.2	0.943
ash, %	25	**1.1 ± 0.02**	**1.2 ± 0.03**	**0.001**
drip loss, %	26	**7.4 ± 0.5**	**9.3 ± 0.6**	**0.022**
pH_u	26	**5.54 ± 0.02**	**5.44 ± 0.02**	**0.001**

[a] Bold font denotes a significant ($p < 0.05$) effect of the fixed factor RN genotype.

DISCUSSION

To our knowledge, this is the first study investigating whether natural variations of HCA precursors in raw pig meat affect the yield of HCAs after cooking. Moreover, this is the first report on HCAs in fried minced patties made of meat originating from animals of different RN genotypes. HCAs were detected in all samples of fried pig meat, and, interestingly, the amounts of PhIP and total HCAs for the two genotypes differed significantly. The levels of MeIQx, 4,8-DiMeIQx, and PhIP (**Table 1**) were in the range of those from other studies where whole pork chops were studied (*28*, *10*).

In an attempt to elucidate which components in pig meat are the most important for the formation of 4,8-DiMeIQx, MeIQx, PhIP, harman, and norharman a principal component analysis (PCA) was performed (**Figure 1**). The bi-plot shows that the

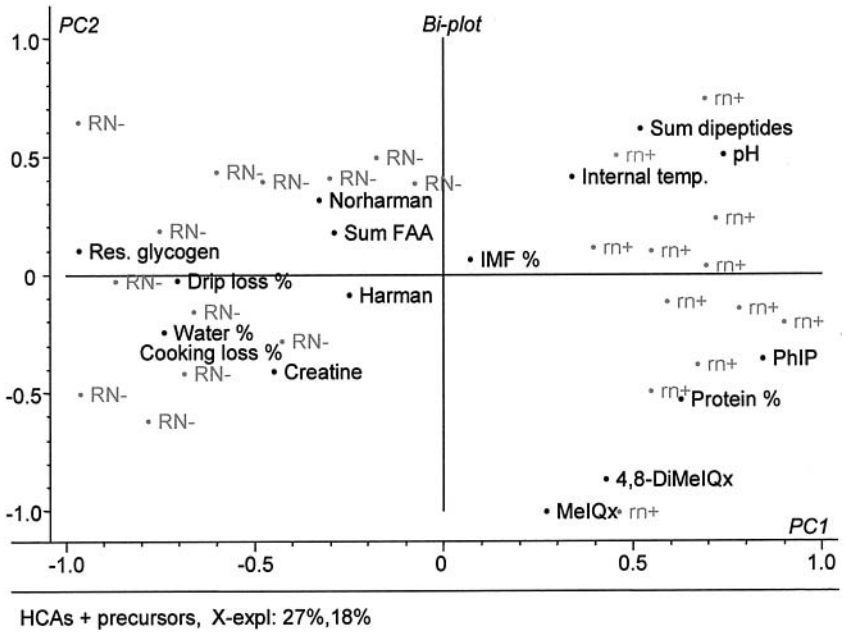

Figure 1. Principal component analysis (PCA) bi-plot. Loadings of measured variables (creatine, residual glycogen, sum of FAAs, protein, IMF, water, pH_u, cooking- and drip-loss of the raw meat, as well as internal temperature and HCAs in the cooked meat). Scores of samples clustered into two groups representing the two RN genotypes.

samples form two well-separated clusters: the carriers of the RN^- allele (denoted RN-) to the left and the noncarriers to the right (denoted rn+). PhIP, and to some extent 4,8-DiMeIQx and MeIQx, covaries along principal component 1 (PC1) with variables such as pH, protein content, and sum of dipeptides (anserine and carnosine) of the meat together with the scores of the noncarriers. The levels of harman and norharman on the other hand, tend to covary with variables that are characteristic of carriers of the RN^- allele, i.e., residual glycogen levels, water content, and drip loss of the meat. The closer to the origin the variables are plotted the less important they are for explaining the variance in the model. In the present model, the explained variance is low (PC1 explains 27% of the variance) but the plot gives a picture of how the most important precursors and HCAs relate to each other. Another important observation in this PCA plot is the clear separation of the two RN genotypes, which suggests that each group might have to be modeled separately.

As mentioned in the Introduction, pigs that are carriers of the dominant RN^- allele are known to have significantly higher levels of glycogen in glycolytic muscles. The exact mechanism behind this glycogen accumulation is not yet known. It could be a result of either a dominant negative mutation inhibiting AMP (adenosine monophosphate) activation and glycogen degradation or a gain-of-function mutation leading to an increased glucose transport and/or glycogen synthesis (*26*). However, the effects of the RN^- allele cause the large variation of the level of residual glycogen, ranging from 7 to 91 μmol/g meat (wet weight) in our study. Our results on the levels of residual glycogen in LD correspond well with recent results obtained in *M. semimembranosus* in another Swedish slaughter pig population (*34*). A residual glycogen value of about 20 μmol/g meat could be regarded as normal in the part of the Swedish slaughter pig population that does not carry the RN^- allele, i.e., in approximately 60% of the total pig population of today (*11*, *19*).

The levels of creatine found in this study agree with creatine levels previously reported in pig meat (*35*, *10*). Similar to the residual glycogen, the content of creatine was higher ($p = 0.010$) in meat from carriers of the RN^- allele than that of normal pigs (**Table 2**). According to Lebret et al. (*36*), the RN^- allele leads to an increase in the relative areas of oxidative type IIA and IIBr fibers in LD. We hypothesized that this shift in myofiber characteristics may lead to differences in the metabolic pattern of LD and may cause the higher level of creatine observed in meat from carriers of the RN^- allele than that of noncarriers. However, as Laser-Reuterswärd (*37*) pointed out, white fiber types have a more rapid energy metabolism, and it could therefore be supposed that the creatine levels should be higher in these fiber types. Obviously, other explanations are plausible and further research on the effect of the RN genotype on creatine content of muscle and meat is needed. No significant differences in creatine concentrations were found between the two sexes or between the two feeding regimes.

Fried meat from carriers of the dominant RN^- allele contained only about half of the total mutagenic HCAs (sum of MeIQx, 4,8-DiMeIQx, and PhIP) found in fried meat from noncarriers. The yield of HCAs would be expected to increase with increasing creatine content, as creatine is a necessary precursor for the formation of HCAs (*38*). Instead, the much higher residual glycogen concentration in the RN^- carriers negatively affected the yield of HCAs. When the level of PhIP was plotted against residual glycogen (**Figure 2**) the plot gave a good illustration of how the samples with low and high residual glycogen are separated into two groups. It is clearly seen that high levels of residual glycogen inhibit the formation of PhIP. Formation of HCAs is influenced by both the levels of precursors and the ratio between them. An optimal effect on the formation of HCAs in model systems has been reported when mono- or disaccharides are present at about half the molar amount of creatine and free amino acids; however, when the concentrations of sugar increased, the formation of mutagens decreased (*38*, *39*). The meat from carriers of the RN^- allele contained, on average, 4-fold higher concentrations of residual glycogen compared with meat from noncarriers (i.e., normal pigs). This may explain the reducing effect that we observed on the total amount of mutagens, PhIP and to some extent MeIQx and 4,8-DiMeIQx, in fried meat from pigs that were carriers of the RN^- allele (**Table 1**). The observed effect of the

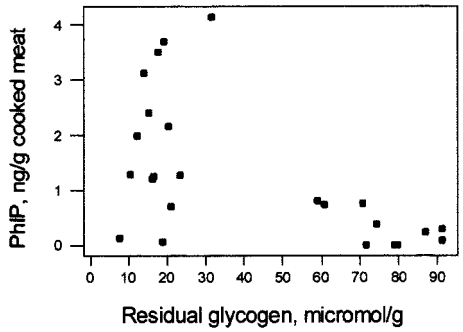

Figure 2. Concentration of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]-pyridine (PhIP) as a function of residual glycogen concentrations in pig meat. Samples with high residual glycogen values represent meat of the carriers of the RN$^-$ allele and those with low residual glycogen values represent meat from noncarriers.

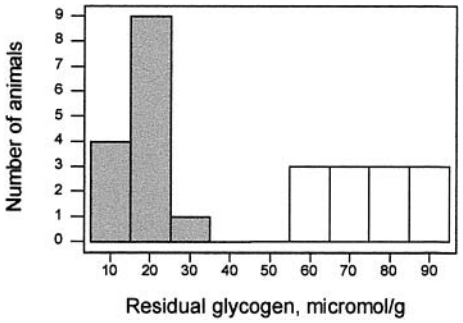

Figure 3. Distribution of the residual glycogen concentration (μmol/g raw meat) of the 26 pig meat samples. Gray bars represent meat from noncarriers, and white bars represent meat from carriers of the RN$^-$ allele.

RN$^-$ allele on the concentration of some other important precursors and modulators (anserine and carnosine, and some FAAs, as well as pH and fatty acid composition of the meat (*40*)) may play a role for the formation of HCAs, but at this stage we cannot isolate this effect from that of the increased residual glycogen values.

Furthermore, our results of concentrations of free amino acids, some of which have previously been shown to produce HCAs when heated in model systems (*7*), were interesting. Generally, our results on levels of free amino acids and dipeptides in porcine LD agree broadly with those of Aristoy and Toldrá (*41*) and Cornet and Bousset (*42*). Using model systems, phenylalanine and creatine have been shown to act as precursors for the formation of PhIP (*43*). In model systems, PhIP has also been formed from creatine heated with leucine, isoleucine, or tyrosine (*7*). Here, we found no correlation between phenylalanine levels in the raw meat and the level of PhIP in the cooked meat, and no correlation between leucine, isoleucine, tyrosine, or any other free amino acid and the PhIP levels. No strong correlations existed between individual FAAs and the level of MeIQx and 4,8-DiMeIQx in the cooked meat.

The comutagens, harman and norharman, occurred in lower concentrations in all fried pig meat samples (LD) than in other types of meat. Harman and norharman have been detected in amounts up to 4.8 and 19.3 ng/g, respectively, in beef steaks fried at 190 °C (*44*), and up to 22 and 30 ng/g, respectively, in grilled bacon strips (*45*). The low levels of harman and norharman in our study might be due to the low levels of tryptophan in pig meat; tryptophan occurred in concentrations below 0.2 μmol/g meat in the pig meat, whereas beef has been reported to contain 1.45 μmol/g (*46*). In a model system, the formation of harman and norharman has been shown to increase with increasing tryptophan concentrations, whereas increasing amounts of creatine or glucose did not affect the yield (*47*). In our study, none of the measured variables were correlated with the level of harman, and the relationships between norharman and urea, ornithine, and phenylalanine in meat from carriers of the RN$^-$ allele need to be studied further.

Placing the different precursors and other factors measured in order of precedence for the formation of the various HCA was difficult in this study. Indeed, our experimental model was not adequate to draw any far-reaching conclusions on the impact of specific precursors on the yield of HCAs. The large impact of the RN$^-$ allele (e.g., the bimodal distribution of the residual glycogen levels (**Figure 3**) with two extreme mean values), makes the model better suited to explain how the RN genotype affects the formation of HCAs.

The most interesting observation in relation to the frying was that a subjective assessment of the degree of surface browning showed that cooked meat from RN$^-$ carriers was significantly darker than that of noncarriers (**Table 3**). The subjective color scores were highly correlated (0.86; $p < 0.001$) with the residual glycogen content of the raw meat. The higher residual glycogen content of meat from carriers of the RN$^-$ allele was probably important for crust color development, presumably leading to a more pronounced Maillard reaction. There was, however, no correlation (or in the case of PhIP, a negative correlation), between the amount of HCAs and the degree of surface browning. This indicates that the degree of surface browning is not a good indicator of HCA formation in pig meat in which the RN$^-$ allele is present.

The cooking loss (34.4 $\pm$ 2.4%, mean $\pm$ SD) is in the range of that from another study in which whole pork chops were grilled to a final internal temperature of 80 °C (*48*) and somewhat higher than that in a Danish study in which ground pork was fried to an internal temperature of 70 °C (*49*). Compared with these studies it can be noted that the mincing of the meat did not considerably affect the water holding capacity during cooking. The cooking loss was larger for the fried meat patties made of meat from RN$^-$ carriers (**Table 3**). The decreased water holding capacity, judged as higher cooking loss in RN$^-$ carriers, is probably due to the combined effect of the RN$^-$ allele on ultimate pH and glycogen and protein levels (*18*, *19*).

CONCLUSIONS

The natural variation of the concentrations of HCA precursors in pig meat was 13-fold for residual glycogen, 1.5-fold for creatine, and 2-fold for both total free amino acids and dipeptides. These variations in the levels of HCA precursors were best explained by the RN genotype of the animals, whereas neither feeding regime nor sex significantly contributed to the variation. The highest levels of residual glycogen were analyzed in meat from pigs that were carriers of the dominant RN$^-$ allele. Increased residual glycogen concentrations significantly reduced the yield of total HCAs, in particular PhIP, and resulted in about 50% lower amounts of total HCAs in cooked meat of RN$^-$ carriers than in cooked meat from normal pigs. Clearly, proper assessment of human intake of HCAs requires further knowledge of the level of HCAs in cooked meat from different species and also on the intra species variation due to genetic factors. The observation that a higher degree of surface browning of fried meat does not necessarily mean higher levels of HCAs is also important for dietary assessment studies.

ABBREVIATIONS USED

HCAs, heterocyclic amines; MeIQx, 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (CAS 77500-04-0); 4,8-DiMeIQx, 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (CAS 95896-78-9); PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (CAS105650-23-5); Norharman, 9H-pyrido[3,4-*b*]indole (CAS 244-63-3); Harman, 1 -methyl-9H-pyrido[3,4-*b*]indole (CAS 486-84-0); FAA, free amino acids; IMF, intramuscular fat; PCA, principal component analysis; LD, M. *Longissimus dorsi*; AMP, adenosine monophosphate.

ACKNOWLEDGMENT

Kristina Andersson is gratefully acknowledged for her contribution to this work by setting up the animal trial and handling all matters connected to producing the pigs. We are very thankful to the Department of Domestic Sciences of Uppsala University and Katarina Virhammar for sharing the temperature-controlled frying device with us; and we would also like to thank Gertrud Andersson, Christina Nilsson, and Eva Borgen for skillful assistance in collecting and analyzing the meat.

LITERATURE CITED

(1) Felton, J. S.; Jägerstad, M.; Knize, M. G.; Skog, K.; Wakabayshi, K. Contents in food, beverages and tobacco. In *Food Borne Carcinogens: Heterocyclic Amines*; Nagao, M., Sugimura T., Eds.; John Wiley & Sons Ltd.: West Sussex, U.K., 2000; pp 31−72.

(2) Wakabayashi, K.; Nagao, M.; Esumi, H.; Sugimura, T. Food-derived mutagens and carcinogens. *Cancer Res., Suppl.* **1992**, *52*, 2092s−2098s.

(3) Nagao, M. A new approach to risk estimation of food-borne carcinogens − heterocyclic amines − based on molecular information. *Mutat. Res.* **1999**, *431*, 3−12.

(4) Sugimura T. Overview of carcinogenic heterocyclic amines. *Mutat. Res.* **1997**, *376*, 211−219.

(5) Sugimura, T. Nutrition and dietary carcinogens. *Carcinogenesis* **2000**, *21*, 387−395.

(6) Totsuka, Y.; Fukutome, J.; Takahashi, M.; Takahashi, S., Tada, A.; Sugimura, T.; Wakabayashi, K. Presence of N2-(deoxyguanosin-8-yl)-2-amino-3,8-dimethylimidazo[4, 5-*f*]quinoxaline (dG-C8-MeIQx) in human tissues. *Carcinogenesis* **1996**, *17*, 1029−1034.

(7) Skog, K.; Johansson, M.; Jägerstad, M. Carcinogenic heterocyclic amines in model systems and cooked foods: a review on formation, occurrence and intake. *Food Chem. Toxicol.* **1998**, *36*, 879−896.

(8) Jägerstad, M.; Laser Reuterswärd, A.; Öste, R.; Dahlqvist, A.; Olsson, K.; Grivas, S.; Nyhammar, T. Creatine and Maillard reaction products as precursors of mutagenic compounds formed in fried beef. In *The Maillard Reaction in Foods and Nutrition*; Waller, G. R., Feather, M. S., Eds.; American Chemical Society: Washington, DC, 1983; pp 507−519.

(9) Vikse, R.; Joner, P. E. Mutagenicity, creatine and nutrient contents of pan fried meat from various animal species. *Acta Vet. Scand*. **1993**, *34*, 1−7.

(10) Pais, P.; Salmon, C. P.; Knize, M. G.; Felton, J. S. Formation of mutagenic/carcinogenic heterocyclic amines in dry-heated model systems, meats and meat drippings. *J. Agric. Food Chem.* **1999**, *47*, 1098−1108.

(11) Enfält, A.-C.; Lundström, K.; Karlsson, A.; Hansson, I. Estimated frequency of the RN$^-$ allele in Swedish Hampshire pigs and comparison of glycolytic potential, carcass composition, and technological meat quality among Swedish Hampshire, Landrace, and Yorkshire pigs. *J. Anim. Sci.* **1997**, *75*, 2924−2935.

(12) Naveau, J. Contribution a l'étude du déterminisme génétique de la qualité de viande poscine. Héritabilité du rendement tecnologique Napole. *Journ. Rech. Porcine Fr. [C.R.]* **1986**, *18*, 265−276.

(13) Le Roy, P.; Naveau, J.; Elsen, J. M.; Sellier, P. Evidence for a new major gene influencing meat quality in pigs. *Genet. Res., Camb*. **1990**, *55*, 33−40.

(14) Fernandez, X.; Tornberg, E.; Naveau, J.; Talmant, A.; Monin, G. Bimodal distribution of the muscle glycolytic potential in French and Swedish populations of Hampshire cross breeds. *J. Sci. Food Agric*. **1992**, *59*, 307−311.

(15) Sayre, R. N.; Briskey, E. J.; Hoekstra, W. G. Comparison of muscle characteristics and post mortem glycolysis in three breeds of swine. *J. Anim. Sci*. **1963**, *27*, 1012−1020.

(16) Monin, G.; Sellier, P. Pork of low technological quality with a normal rate of muscle pH fall in the immediate post-mortem period: the case of the Hampshire breed. *Meat Sci*. **1985**, *13*, 49−63.

(17) Estrade, M.; Vignon, X.; Monin, G. Effect of the RN$^-$ gene on ultrastructure and Protein fractions in pig muscle. *Meat Sci*. **1993**, *35*, 313−319.

(18) Lundström, K.; Andersson, A.; Hansson, I. Effect of the RN gene on technological and sensory meat quality in crossbred pigs with Hampshire as terminal sire. *Meat Sci*. **1996**, *42*, 145−153.

(19) Lundström, K.; Enfält, A.-C.; Tornberg, E.; Agerhem, H. Sensory and technological meat quality in carriers and noncarriers of the RN$^-$ allele in Hampshire crosses and in pure-bred Yorkshire pigs. *Meat Sci.* **1998**, *48*, 115−124.

(20) Johansson, L.; Lundström, K.; Jonsäll, A.; Lundh, T. Effects of red clover silage and aging time on sensory characteristics and cooking losses on loin (*M. longissimus dorsi*) from Hampshire crosses with and without the RN$^-$ allele. *Food Qual. Pref.* **1999**, *10*, 299−303.

(21) KRAV. *KRAV Standards*. Uppsala, Sweden, 2000.

(22) Enfält, A.-C.; Lundström, K.; Hansson, I.; Johansen, S.; Nyström, P.-E. Comparison of noncarriers and heterozygous carriers of the RN$^-$ allele for carcass composition, muscle distribution and technological meat quality in Hampshire-sired pigs. *Livest. Prod. Sci.* **1997**, *47*, 221−229.

(23) Enfält, A.-C.; Lundström, K.; Hansson, I.; Lundeheim, N.; Nyström, P.-E. Effects of outdoor rearing and sire breed (Duroc or Yorkshire) on carcass composition and sensory and technological meat quality. *Meat Sci.* **1997**, *45*, 1−15.

(24) Lundström, K.; Enfält, A.-C. Rapid prediction of phenotype in pigs by means of meat juice. *Meat Sci.* **1997**, *45* 127−131.

(25) Dalrymple, R. H.; Hamm, R. A method for extraction of glycogen and metabolites from a single muscle sample. *J. Food Technol.* **1973**, *8*, 439−444.

(26) Milan, D.; Jeon, J.-T.; Looft, C.; Amarger, V.; Robic, A.; Thelander, M.; Rogel-Gaillard, C.; Paul, S.; Iannuccelli, N.; Rask, L.; Ronne, H.; Lundström, K.; Reinsch, N.; Gellin, J.; Kalm, E., Le Roi, P.; Chardon, P.; Andersson, L. A mutation in PRKAG3 associated with excess glycogen content in pig skeletal muscle. *Science* **2000**, *288*, 1248−1251.

(27) Wahlefeld, A. W.; Holz, G.; Bergmeyer, U. Creatine. In *Methods of Enzymatic Analysis*; Bergmeyer, U., Ed.; Verlag Chemie Weinheim & Academic Press: New York and London, 1974; vol. 4, pp 1786−1790.

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

(29) Gross, G. A.; Grüter, A. Quantification of mutagenic/carcinogenic heterocyclic aromatic amines in food products. *J. Chromatogr*. **1992**, *592*, 271−278.

(30) Fay, L. B.; Ali, S.; Gross, G. A. Determination of heterocyclic aromatic amines in food products: automation of the sample preparation method prior to HPLC and HPLC−MS quantification. *Mutat. Res.* **1997**, *376*, 29−35.

(31) Johansson, M.; Jägerstad, M. Influence of oxidized deep-frying fat and iron on the formation of food mutagens in a model system. *Food Chem. Toxicol.* **1993**, *31*, 971−979.

(32) Minitab. Minitab Users Guide, Release 12. Minitab Inc.: State College, PA, 1998.

(33) Unscrambler, Multivariate analysis − in practice. A training package. CAMO A.S.A.: Oslo, Norway.
(34) Josell, Å.; Martinsson, L.; Borggaard, C.; Andersen, J. R.; Tornberg, E. Determination of RN$^-$ genotype in pigs at slaughter-line using visual and near-infrared spectroscopy. *Meat Sci.* **2000**, *55*, 273−278.
(35) Klont, R. E.; Lambooy, E. Effects of preslaughter muscle exercise on muscle metabolism and meat quality studied in anaesthetized pigs of different halothane genotypes. *J. Anim. Sci.* **1995**, *73*, 108−117.
(36) Lebret, B.; Le Roy, P.; Monin, G.; Lefaucheur, L.; Caritez, J. C.; Talmant, A.; Elsen, J. M.; Sellier, P. Influence of the three RN genotypes on chemical composition, enzyme activities, and myofiber characteristics of porcine skeletal muscle. *J. Anim. Sci.* **1999**, *77*, 1482−1489.
(37) Laser Reuterswärd, A.; Skog, K.; Jägerstad, M. Mutagenicity of pan-fried bovine tissues in relation to their content of creatine, creatinine, monosaccharides and free amino acids. *Food Chem. Toxicol.* **1987**, *25*, 755−762.
(38) Skog, K.; Jägerstad, M. Effects of monosacharides and disacharides on the formation of food mutagens in model systems. *Mutat. Res.* **1990**, *230*, 263−272.
(39) Skog, K.; Jägerstad, M.; Laser Reuterswärd, A. Inhibitory effect of carbohydrates on the formation of mutagens in fried beef patties. *Food Chem. Toxicol.* **1992**, *30*, 681−688.
(40) Nilzén, V.; Babol, J.; Dutta, P. C.; Lundeheim, N.; Enfält, A.-C.; Lundström, K. Free range rearing of pig with access to pasture grazing − effect on fatty acid composition and lipid oxidation products. *Meat Sci.* **2001**, *58*, 267−275.
(41) Aristoy, M.-C.; Toldrá, F. Concentration of free amino acids and dipeptides in porcine skeletal muscles with different oxidative patterns. *Meat Sci.* **1998**, *50*, 327−332.
(42) Cornet, M.; Bousset, J. Free amino acids and dipeptides in porcine muscles: differences between red and white muscles. *Meat Sci.* **1999**, *51*, 215−219.
(43) Felton, J. S.; Knize, M. G. Mutagen formation in muscle meats and model heating systems. In *Mutagens in Food: Detection and Prevention*; Hayatsu, H., Ed.; CRC Press: Boca Raton, FL, 1991; pp 57−66.
(44) Gross, G. A. Simple methods for quantifying mutagenic heterocyclic amines in food products. *Carcinogenesis* **1990**, *11*, 1597−1603.
(45) Gross, G. A.; Turesky, R. J.; Fay, L. B.; Stillwell, W. G.; Skipper, P. L.; Tannenbaum, S. R. Heterocyclic aromatic amine formation in grilled bacon, beef and fish and in grill scrapings. *Carcinogenesis* **1993**, *14*, 2313−2318.
(46) Arvidsson, P.; van Boekel, M. A. J. S.; Skog, K.; Jägerstad M. Kinetics of formation of polar heterocyclic amines in a meat model system. *J. Food Sci.* **1997**, *62* (5), 911−916.
(47) Skog, K.; Solyakov, A.; Jägerstad, M. Effects of heating conditions and additives on the formation of heterocyclic amines with reference to amino-carbolines in a meat juice model system. *Food Chem.* **2000**, *68*, 299−308.
(48) Sheard, P. R.; Wood, J. D.; Nute, G. R.; Ball, R. C. Effects of grilling to 80 °C on the chemical composition of pork loin chops and some observations on the UK Natural Food Survey estimate of fat consumption. *Meat Sci.* **1998**, *49*, 193−204.
(49) Aagaard Nielsen, P.; Vahl, M.; Gry, J. HPLC profiles of mutagens in ground pork fried at different temperatures. *Z.-Lebensm.-Unters Forsch.* **1988**, *187*, 451−456.

Received for review September 24, 2001. Revised manuscript received January 28, 2002. Accepted February 21, 2002. We are grateful for financial support from the Swedish Cancer Foundation, Mistra's Food 21 program, and the Swedish Council for Forestry and Agricultural Research. The study has also been financially supported by the Commission of the European Communities, specific RTD program Quality of Life and Management of Living Resources, QLK1-CT99-001197, Heterocyclic Amines in Cooked Foods − Role in Human Health. It does not necessarily reflect views and in no way anticipates the Commission's future policy in this area.

JF011239H