BEEF AGEING AND FORMATION OF HETEROCYCLIC AMINES IN GRILLED STEAKS

Polak, Tomaž[1]; Gašperlin, Lea[1]; Andrenšek, Samo[2]; Žlender, Božidar[1]

[1] University of Ljubljana, Biotechnical Faculty, Department of Food Science and Technology, Jamnikarjeva 101, SI-1111 Ljubljana, Slovenia, E-mail: tomaz.polak@bf.uni-lj.si

[2] National Institute of Chemistry, Laboratory for Food Chemistry, Hajdrihova 19, SI-1000 Ljubljana, Slovenia

Background

Grilling of meat and fish products may generate low ppb levels of mutagenic/carcinogenic heterocyclic amines (HAs). Many heterocyclic amines are formatted via the Maillard reaction from creatine, creatineine, free amino acids and monosaccharides; compounds naturally occurring in protein-rich foods of animal origin. The formation and amount of HAs are dependent on physical parameters, such as cooking temperature and time, cooking technique and equipment, heat and mass transport, and on chemical parameters, especially the precursors of HAs. The most common HAs in cooked foods are 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx) and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), which belongs to mutagens in Ames test and shows carcinogenicity in animal testing (Skog, 1998; Taylor, 1984). Ageing or conditioning of beef is normal procedure with unprocessed meat associated with an increase in tenderness and flavor. In the case of increased amount of precursors of HAs arising during meat ageing e.g. free amino acids, it may cause increased formation of some HAs during cooking. Our hypothesis is that the stage of beef ageing increases the amount of HAs in cooked meat, because of increase of precursors especially free amino acids.

Objectives

The objective of this study was to examine the relation between stage of ageing beef meat measured as free amino acids content and formation of heterocyclic amines in grilled beef (*m. longissimus dorsi*- LD).

Methods

Eight Simmental bulls aged from 19 to 20 months were slaughtered in an industrial slaughterhouse and normally chilled. 24 hours after slaughter eight LD muscles were excised and divided into 3 portions, vacuum packed and conditioned/aged at 1°C for 0 (non-aged sample), 2 and 4 weeks (aged samples). After ageing the samples were frozen and stored at -21°C till analyses.

Samples for determination of free amino acids were homogenized, then extracted and analyzed with amino-analyzer (Cordoba, 1994). Extraction recovery rates were determinate by the addition of a known amount of L-norleucine (internal standard), and free amino acids were identified and quantified using retention time and spectra from standards of known concentration, run under the same conditions.

Beef LD-steaks were grilled to a core temperature T_c= 80 °C on both sides simultaneously using double-plate teflon coated heating device. The size of steaks was 10 cm × 5 cm × 2.5 cm. No salt or oil was added. The temperature of heating plates was 220°C.

About two millimeters thick outer layers of the grilled meat were extracted and analyzed for HAs using reverse-phase HPLC (Murkovic *et al.*, 2000; Borgen *et al.*, 2001). Extraction recovery rates were determinate by the addition of a known amount of HAs (internal standard), and HAs were identified and quantified using retention time and spectra from synthetic standards of known concentration, run under the same conditions.

The data were statistically analyzed by the method of the least squares using the GLM procedure (SAS, 1990). The statistical model for HAs content of beef meat includes the effects of conditioning/ageing (non-aged, 2 and 4 weeks aged).

Results and discussion

Total free amino acids content (table 1) significantly increased ($P<0.0001$) and redoubled in beef LD muscle during 4 weeks ageing (19,74 vs. 38.45 µmol/g). After Taylor (1984) increase in free amino acids increases mutagens formation.

Table 1 and figure 1 shows the amount of HAs formed in beef steaks aged different time. MeIQx and PhIP were detected in all samples. The rate of ageing influences on the amount of free amino acids and indirect increases the amount of MeIQx and PhIP. MeIQx significantly increases ($P\leq0.01$) after 4 weeks ageing and PhIP increases significantly ($P\leq0.001$) already after two weeks of ageing. These results confirm our hypothesis that with increased stage of beef ageing also increases formation of heterocyclic aromatic amines in grilled beef prepared to relatively high core temperature of 80°C.

Conclusions

The amount of HAs in beef meat depends on rate of ageing.

The largest amount of HAs was formed in beef aged 4 weeks.

The rate of ageing highly increases PhIP from 0,046 ng/g at 0 week to 0,213 ng/g at 4 weeks.

Pertinent literature

Borgen, E., Solyakov, A., Skog, K. Effects Of Precursor Composition And Water On The Formation Of Heterocyclic Amines In Meat Model Systems (2001). Food Chemistry, 74(1):11-19.

Cordoba, J.J., Rojas, T.A., Gonzalez, C.G. Evolution of Free Amino-Acids and Amines During Ripening of Iberian Cured Ham, Journal Agr. Food Chem (1994). 42: (10) 2296-2301.

Murkovic, M., Pfannhauser, W. Analysis of the cancerogenic heterocyclic aromatic amines in fried meat. (2000). Fresenius J. Anal. Chem. 366:375-378.

Skog, K., Johansson, M., Jägerstad, M. Carcinogenic Heterocyclic Amines In Model System And Cooked Foods: A Review On Formation, Occurrence and Intake. (1998). Food and Chemical Toxicology 36: 879-896.

Taylor, R., Fultz, E., Shore, V. Mutagen Formation In A Model Beef Boiling System I. Conditions With A Soluble Beef-Derived Fraction. (1984). J. Environ. Sci. Health. A19(7):791-817.

Table 1. HAs in beef steaks after different time of conditioning/ageing.

Parameters	Time of ageing (weeks)			Significance of effect (P-value)
	0 (n=8)	2 (n=8)	4 (n=8)	
Total free amino acids (µmol/g)	19,748±3,009	29,941±5,229	38,457±4,208	<0,0001
MeIQx (ng/g)	0,163[a]±0,049	0,167[a]±0,053	0,210±0,024	0,0065
PhIP (ng/g)	0,046±0,049	0,119±0,051	0,213±0,051	<0,0001

[a] Means with the same letter were not significantly different (P> 0.05);
n – number of repetition

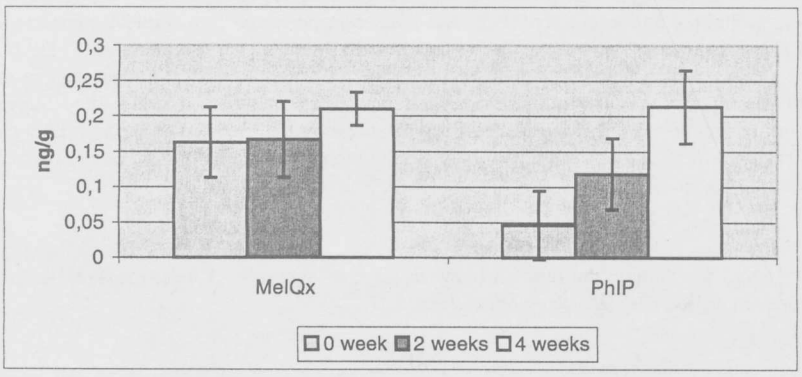

Figure 1. HAs in beef stakes after different time of ageing.