EFFECT OF FLAVOUR ENHANCERS ON THE FORMATION OF HETEROCYCLIC AMINES IN FRIED BEEF PATTIES

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Abstract— **Heterocyclic aromatic amines (HAA) are carcinogenic process contaminates found in the crust of fried meat. The objective was to examine the effect of application of flavour enhancers (monosodium glutamate, disodium 5-inosinate, and two commercial products, Super YE (yeast extract) and Super RK (a processed flavour) on the formation of HAA, colour and sensory quality in fried beef patties. After frying of treated and untreated patties, concentration of four HAA were analysed by HPLC. Concentrations of the mutagenic MeIQx and PhIP in untreated patties ranged from 0.1-0.4 ng/g and 0.1-0.2 ng/g, respectively while the content of co-mutagenic ß-carbolines Norharman and Harman varied between 0.2-3.2 ng/g and 0.2-7.8 ng/g, respectively. The addition of the flavour enhancer to beef patties resulted in extensive changes of the concentrations of HAA. Yeast extract reduced the content of MeIQx by about 50%, while sodium glutamate and processed flavour resulted in an increase of 75-100%. In particular, the concentrations of ß-carbolines increased by a factor of 10 - 40 with application of the processed flavour. Application of all flavour enhancers improved sensory evaluation scores compared to controls.**

Index Terms—**Heterocyclic Aromatic Amines, flavour enhancer, flavouring, glutamate, sensory test, beef patties.**

I. INTRODUCTION

Epidemiological studies have shown evidence that daily diets could be responsible for the initiation of different kinds of cancer. While searching for a possible relationship between diet and cancer, Heterocyclic Aromatic Amines (HAA) - process contaminates present in heated food - were identified. These substances can be found in particularly high concentrations in the crust of fried, broiled and cooked meat and fish. These compounds were shown to be mutagenic in the Ames test with *Salmonella* Thyphimurium TA 98 with an S9 mix and carcinogenic in long-term animal studies that had been conducted on monkeys (Adamson et al., 1990). The International Agency for Research on Cancer classified several HAA as possible or probable carcinogens and recommended to reduce their dietary intake (IARC, 1993). The major HAA in fried meat products are MeIQx, 4,8-DiMeIQx and PhIP. These substances are responsible for most of the observed mutagenic activity in foods and are usually formed as products of the Maillard reaction. Their precursors are creatine or creatinine, free amino acids and sugars. Key parameters that influence HAA formation are temperature and heating time (Arvidsson, Van Boekel, Skog & Jagerstad, 1997). Moreover, mass transport of precursors from the interior of meat or fish products to product surfaces affects the formation of HAA. In addition to these physical parameters, recent studies have shown that addition of antioxidants such as tocopherols or natural extracts with radical scavenging properties can inhibit the formation of HAA (Gibis, 2007, Gibis & Weiss, 2010). The influence of other typical meat and fish product constituents has however not yet been investigated. Since flavour enhancers are often used in the manufacture of meat of fish products to enhance sensory properties, information on the effect of their presence on formation of HAA is needed. The aim of this study was therefore to investigate the effect of addition of different flavour enhancer including monosodium glutamate, disodium 5-inosinate, and two commercial flavour enhancers (Super YE and Super RK) on the formation of HAA in fried beef patties. The sensory characteristics of treated and fried patties were evaluated for odour, taste, colour, and meat flavour.

II. MATERIALS AND METHODS

Preparation of Beef Patties: Roughly desinewed and defatted beef was coarsely minced with a grinder having a 3 mm perforated plate. 1.2% salt, 0.05% black pepper and flavour enhancers (**Table 1;** Ajinomoto, Hamburg, Germany) and/or glutathione (Sigma-Aldrich, Taufkirchen, Germany) were added to the minced meat. The mixture was then homogenized using a blender and 60g ± 1g of the homogenized mixture formed into patties (1.2 cm thick x 8.5 cm diameter) using a patty mould. Controls consisted of minced meat void of flavour enhancers.

Frying of Beef Patties: A double contact grill (Nevada, Neumärker, Hemer, Germany) was heated to 230 °C. Beef patties were placed between two tin foils, which had been lightly coated with sun flower oil, and fried on the double contact plates grill for 2:45 min. Core and surface temperatures of beef patties during frying were recorded with a data logger (Ahlborn, Holzkirchen, Germany). The contact grill frying led to beef patties having a core temperature of 72 °C and a surface temperature < 190°C by the end of the frying process. Per batch, a total of eight patties were fried and then mixed together for chemical analysis.

Table 1. Type and concentration of added flavour enhancers.

Flavour enhancer	Added concentrations in g/kg		
Mono sodium glutamate	2.5	5	10
Disodium 5-inosinate	0.25	0.5	1
Yeast extract (Super YE)	3	6	
Processed flavour (Super RK)	3	5	

Determination of HAA: Samples were analyzed for 15 polar and apolar HAA. A previously published, modified HPLC method (Gibis, 2007) based on the method described by Gross and Grueter (1992) was used. Presence and concentration of HAA, as well as the β-cabolines Norharman and Harman, in the samples (n=4) were identified by comparing the retention times and UV-spectra with standards. The quantification was carried out with the method of standard addition.

Sensory evaluation: A trained sensory panel consisting of students or sensory experts was used to assess the sensory properties of samples. Panellists were trained with fried beef patties that were free of or contained flavour enhancers. After frying, beef patties were served hot to the testers. The sensory panellists (n=15, n=16) were asked to evaluate the beef patties using a hedonic rank ordering test ($\alpha = 0.05$). The most pleasant sample was given a rank of 1 while the least acceptable products were given a rank of 3 or 4 according to the number of tested batches. If the testers did not find a difference between samples, they calculate respectively the mean rank of the samples with the same ranks.

Colour measurement: Colour of patties was determined by a Chroma Meter CR 200 (Minolta, Osaka, Japan). L*- (brightness), a*- (+red to -blue), and b* - (+yellow to - green) values of patties were measured 1 hour after heating. Of every batch, eight patties were each measured 3 times.

Statistical analysis: Instrumentally measured data (concentrations of HAA, colour of patties) were subjected to an analysis of variance using the GLM and Tukey-Test ($\alpha=0.05$) using SAS (Version 9.0, SAS Institute INC., Cary, U.S.A.). For sensory test, a Friedman test was used to analyze for significant differences of the rank sums ($\alpha=0.05$).

III. RESULTS AND DISCUSSION

In all samples, including the controls, two mutagenic HAA, MeIQx and PhIP and the co-mutagenic β-carbolines Norharman and Harman were detected (**Fig. 1** and **2**). In comparison to the control, the addition of monosodium glutamate resulted in a significant increase in the concentration of MeIQx ($p<0.01$) of approximately 100% in fried beef patties. Glutamate concentration had little effect on formation of MeIQx, that is samples that contained different concentrations of glutamate (2.5, 5 and 10 g/kg) did not differ significantly ($p>0.05$) in their concentration of MeIQx. A significant reduction of MeIQx of approximately 50% could only be observed upon addition of yeast extract ($p<0.05$). The content of PhIP in samples was generally very low (0.05-0.1 ng/g in all batches) and close to the detection limit of the analytical method, making a statistical analysis of PhIP results impractical. A marginal increase of concentrations of Norharman and Harman was found with the application of the two commercial flavour enhancers, Super-RK (the processed flavour) and Super YE (the yeast extract) (**Fig. 2**). Norharman and Harman were formed at lower temperatures during the manufacturing of processed flavours and the formation of co-mutagenic β-carbolines is independent from available occurrence of creatine and creatinine (Pfau & Skog, 2004).

Due to the surprising results with Super-YE, the yeast extract, a second set of experiments was conducted. Super-YE contained according to manufacturer's specification more than 2.5% reduced glutathione in addition to glutamic acid. Glutathione is known to possess a high antioxidant potential. In its reduced form, the sulfhydryl group of cysteine is able to donate a hydrogen ion and electron ($H^+ + e^-$) that may inhibit HAA-formation (Schoch, Gibis & Fischer, 2001). We thus hypothesized that suppression of formation of HAA may be due to the presence of reduced glutathione in the yeast extract. To test this hypothesis, reduced glutathione was directly added to ground meat (0.15 g/kg) and HAA formation measured in fried patties. The concentration of glutathione used was equal to the highest content of yeast extract tested. Results showed indeed that HAA formation was inhibited by reduced glutathione during frying of patties, suggesting that the inhibitory effect of the yeast extract is likely due to the presence of reduced glutathione (**Fig. 1**).

Colour measurements (**Fig. 3**) demonstrated that the control samples had the highest a*-values and significantly differed from all other samples with exception of the two batches that contained yeast extract and the highest concentration of the processed flavour Super-RK. Moreover, the L*- values are higher for controls and yeast extracts. More free amino acids likely caused to an increase in concentrations of Maillard products (Schoch et al., 2001).

Finally, the sensory rank sum tests demonstrated that addition of flavour enhancers significantly often improved the acceptance of the beef patties regardless of which flavour enhancer was used. Sensory ranks sums were frequently significantly lower for treated beef patties than for controls (**Fig. 4**). In particular, panellists often reported that the characteristic meat flavour was enhanced and patties were significantly more aromatic than controls ($p>0.05$). After frying, the three batches that contained mono sodium glutamate substantially more favourably received by the sensory testers with respect to meat flavour when compared to control.

IV. CONCLUSION

The addition of flavour enhancers generally enhanced the formation of β-carbolines Norharman and Harman in fried

beef patties. Addition of mono sodium glutamate resulted in substantial increases in the concentrations of two carcinogenic HAA, namely MeIQx and PhIP. On the other hand, the commercial flavour enhancer on yeast extract basis led to reductions in HAA content which could be attributed to the presence of reduced glutathione in the extract. Tested flavour enhancers had often significantly better sensory evaluation scores when compared to control samples. Results therefore suggest that manufactures wishing to use flavour enhancers to improve quality of fried meat products should assess the potential of such compounds to enhance formation of dangerous mutagenic compounds. Our studies also suggest that either use of yeast extracts that naturally contain an HAA formation inhibitor such as reduced glutathione or direct addition of reduced glutathione to product containing flavour enhancers may potentially mediate the negative effects of the added flavour enhancers.

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FIGURES

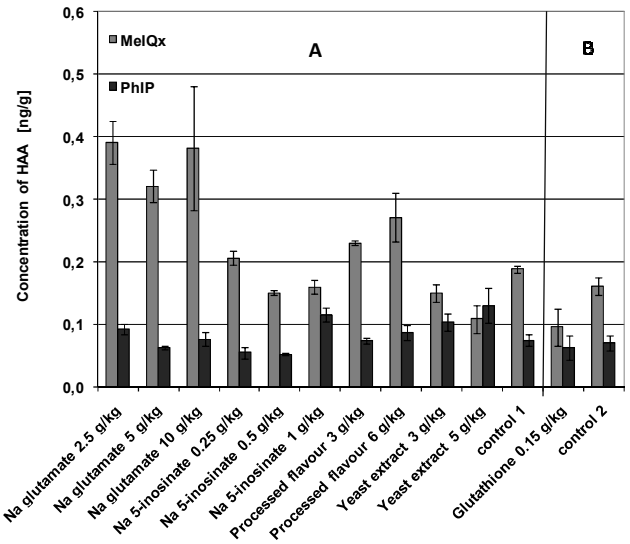

Fig. 1 Concentration of MeIQx and PhIP after application of different flavour enhancer in fried beef patties

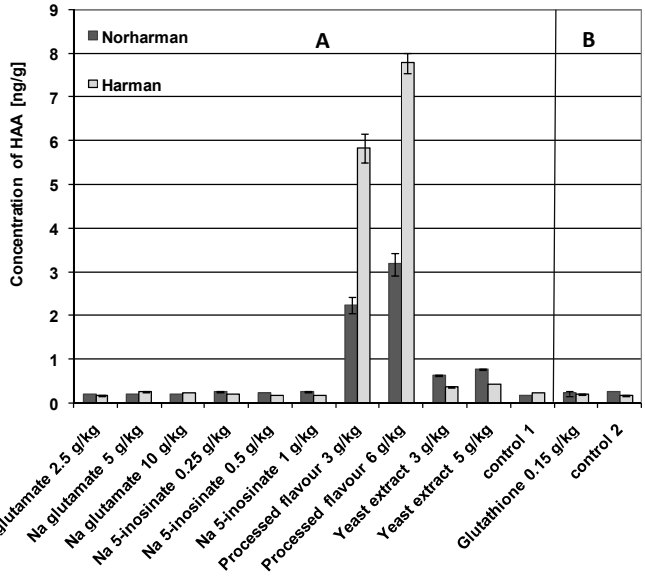

Fig. 2 Concentration of β-carbolines after application of different flavour enhancer in fried beef patties (Experiments A and B with different raw material)

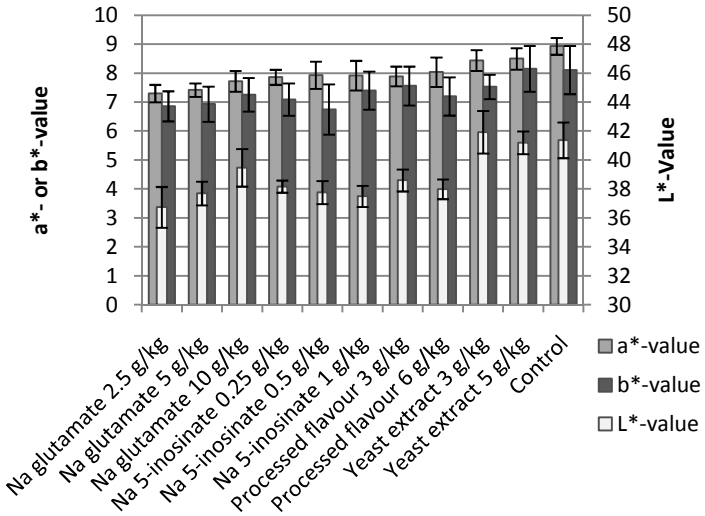

Fig. 3 Colour measurement (L*-,a*-, and b*-values) of fried beef patties after application of different flavor enhancers and replacers

ABBREVIATIONS

HAA - Heterocyclic Aromatic Amines,
MeIQx - 2-amino-3,8-dimethylimidazo[4,5-*f*] quinoxaline,
4,8-DiMeIQx - 2-amino-3,4,8-trimethylimidazo [4,5-*f*]quinoxaline,
PhIP - 2-amino-1-methyl-6-phenylimidazo[4,5-*b*] pyridine,
Norharman - 9*H*-pyrido[3,4-*b*]indole,
Harman - 1-methyl-9*H*-pyrido[3,4-*b*]indole.

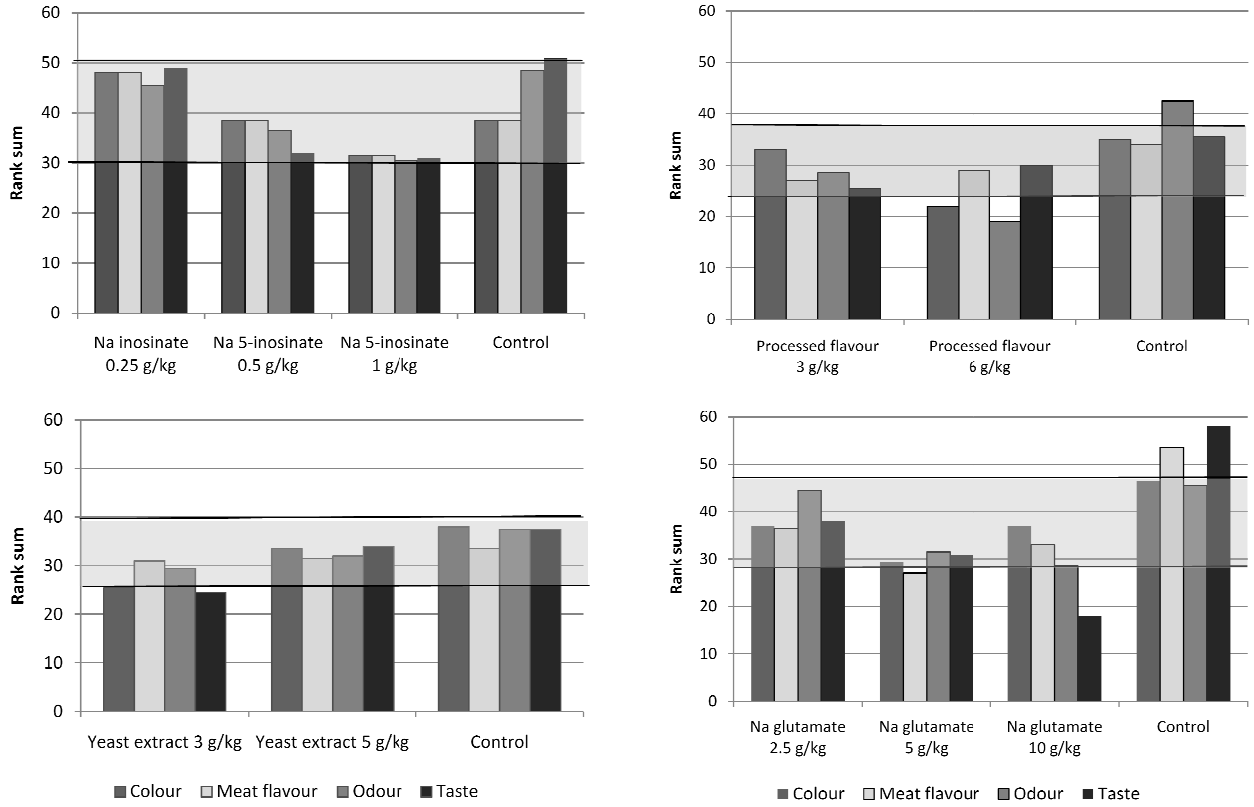

Fig. 4 Sensory rank ordering test of fried beef patties after addition of flavour enhancers and flavours (α=0.05, n=16 significant <25 (30) or > 39(50) rank sum, n=15 significant <23 (28) or > 37(47) rank sum)

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