

## TOXICOLOGICAL AND SENSORIAL BENEFIT OF CYSTEINE ADDITION TO HAMBURGER

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**Keywords:** heterocyclic amines, cysteine, IQx, MeIQx, Hamburger**BACKGROUND:**

Thermal processing of meat often enable products to be made edible, make them more appetizing or provide microbiological stability during storage. However, in recent years consumers have been feeling more insecure about consumption of meat since researchers have pointed out that there is probably an increased risk in the development of different kinds of cancer by eating red meat [1]. This is due to the fact that during household cooking preparation of meat the natural components creatine/-ine, amino acids and sugars can form mutagenic and carcinogenic compounds which chemically belong to the heterocyclic amines (HAs) [2]. Consequently, many institutions have made recommendations to change dietary habits on the eating of meat. The American Institute For Cancer Research, for instance, recommended not to consume burnt or charred food, especially meat and fish, and to avoid grilling foods over a direct flame, as well as frying or boiling in excess [1]. Since the high mutagenic potency of the HAs has been established, scientists all over the world made efforts to lower HA formation and to decrease mutagenic activity by the variation of preparation methods or the addition of substances like antioxidants [3, 4].

**OBJECTIVES:**

Following our previous research where we found that the SH-containing substances glutathion, L-cysteine and N-acetylcysteine offer a great inhibitory effect on HA formation in different model systems [5], we used L-cysteine and L-cysteine hydrochloride monohydrate in the preparation of hamburgers. Besides this principal mechanism, L-cysteine is known to produce meat-like flavour compounds during heating with sugars [6]. For this reason, in addition to the analytical HA determination we made a sensory evaluation of the products.

**METHODS:**

**Chemicals and Materials:** L-cysteine p.a. was purchased from Merck, Darmstadt Germany and L-cysteine hydrochloride monohydrate was a sample from BFGoodrich, Munich, Germany. Beef was purchased from a local wholesaler, cut into 2x2 cm pieces and minced through a 9 and 3 mm grinding plate. Meat was stored until used at -20 °C. The chemical analysis data were : water 69.0 %; fat 10.3 %; Protein 20.3 % (connective tissue: 3.2 %) and ash 1.00 %. For methods see [7].

**Preparation of Hamburger:** Batches of 1 kg minced meat, 13 g NaCl and 0 g; 0.5 g; 1.0 g or 1.5 g of the cysteine compound were mixed for 3 minutes with a Kitchen Aid, (Hobart, Troy, USA). Portions of 80 ± 0.5 g meat were formed with a hamburger former into patties of 10 cm diameter and 16 mm high. Patties were heated with a double-sided contact plate heating device (Nevada, Neumärker, Germany) for 5 minutes at a surface temperature of heating plates of 230 °C. These heating conditions have been shown in pretests to produce the most appetizing burgers for the sensory panel. 4 burgers were heated in parallel on tin aluminium foil (with little oil) to avoid contaminating the following batches. Hamburgers were stored at -20 °C until sensory evaluation and analytical HA determination.

**Sensory evaluation:** Our sensory test panel of 30 persons were recruited from students and scientists of our institute. Each person got a plate with coded numbers of 4 pieces of Burgers and a form to write down the results. For each sample the sensory panel made a comment on colour (data not shown), smell and taste by putting a mark on a 10 cm bar from dislike (at 0 cm) to like (at 10 cm). For evaluation, the place of the mark on the bar was measured with a ruler up to one decimal place. This value was used for statistical analysis performed by SAS software.

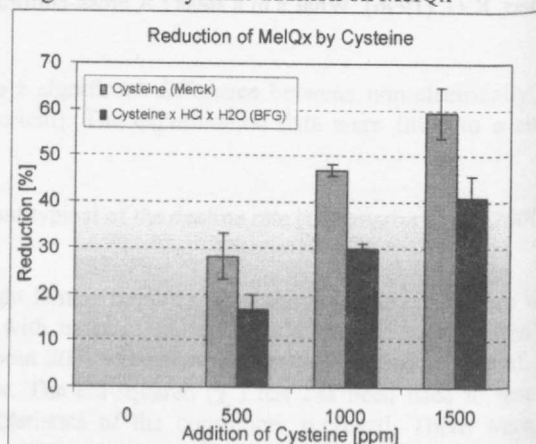
**Analytical HA determination:** Sample cleanup procedure and analytical HPLC determination was performed according to the method of Gross and Grüter [8].

**RESULTS AND DISCUSSIONS:**

The hamburgers of different trials which were heated without the addition of cysteine contained 0.64 ng/g MeIQx (S.D. 0.08), 0.21 ng/g 4,8-DiMeIQx (S.D. 0.04) and 0.49 ng/g PhIP (S.D. 0.14). Furthermore, we could identify the co-mutagenic  $\beta$ -carbolines norharman and harman in samples with cysteine addition as well as in the control samples. These amounts are in accordance with values found in newer literature [2] or representing real household or restaurant cooking styles [9].

As shown in Figure 1, the addition of 500 ppm to 1500 ppm of chemical grade cysteine to hamburgers causes a significant, dose dependant decrease in MeIQx formation. This decrease of up to 70% is in good agreement with our previous studies showing a significant reduction of MeIQx in aqueous as well as in meat matrix based model systems [5]. The inhibiting effect of cysteine-hydrochloride-monohydrate seems to be about one third lower than that of cysteine. This effect, however, could be explained by comparing the molecular weight of 175.6 g/mol for Cys x HCl x H<sub>2</sub>O and 121.2 g/mol for cysteine which is also about one third lower. We conclude, therefore, that the inhibitory potency of cysteine-

Fig.1 Effect of cysteine addition on MeIQx



hydrochloride monohydrate is as powerful as that of cysteine. Our study corresponds to that of Trompeta and O'Brien [3], who show that cysteine can effectively inhibit mutagen formation in an aqueous model system when tested in the Ames assay. The mechanism behind this suppressing effect might rest in the presence of a free sulfhydryl group which can participate as a reducing agent directly in Maillard reactions or act as a scavenger of free radical species [10].

Model systems show that characteristic aromas of cooked meat can be formed when Maillard reactions between cysteine and ribose take place [6]. Beside smaller quantities of free ribose, the ATP derived inosine monophosphate (IMP) is the main source of ribose in meat. We expect therefore, that the addition of cysteine might have a beneficial effect on flavour development of hamburger products. Figure 2 presents the sensory assessment of smell and taste for burgers supplemented with cysteine hydrochloride monohydrate, which offer in principle, the same results as for the trial with cysteine addition. As the width of the standard deviation indicates, the individual preference between the 30 test persons of our sensory panel differ in a wide range for both CysxHClxH<sub>2</sub>O supplemented and control burgers. Statistical analysis with the Tukey test result in no significant differences ( $p < 0.05$ ) between the samples, neither in the assessment of smell nor in taste. The mean values for smell are all in the area of pleasant (5 - 10) with a trend for 500 and 1000 ppm supplemented burgers to be more liked than the control sample. This tendency was also repeated in the assessment of taste where the burgers supplemented with low and middle doses were slightly more enjoyed than the control burgers. Interestingly, the burgers with high supplement levels (1500 ppm) ranging from the level of the control burger seem to offer no sensorial advantage or disadvantage. Furthermore, the colour assessment of the supplemented products (data not shown) indicates that the addition of cysteine lead to lighter products, which demonstrate the ability of cysteine to block nonenzymatic browning reactions as described before [10].

Practical use of L-cysteine hydrochloride monohydrate in industrial production of beef patties will not be limited by economic factors because the used quantity of 1g per kg meat costs only about US \$ 0.02. Furthermore, the U.S. Food and Drug Administration allows the use of L-cysteine hydrochloride monohydrate for nutritive proposes up to 2.3 percent by weigh of total protein [11], while the use in Europe is only permitted to improve technological characteristics of wheat dough.

## CONCLUSIONS:

This study clearly demonstrates the inhibiting potential of L-cysteine and L-cysteine hydrochloride monohydrate on the formation of mutagen/carcinogen MeIQx in hamburgers cooked in an appetizing style. These findings are in good agreement with our previous studies also showing a suppressive effect of organosulfur compounds on HA formation.

The sensory evaluation of the products indicate that there might also be an improvement in smell and taste but not in a significant way. Therefore it will be an interesting challenge in the future not only to focus on the aim of lowering thermally generated process mutagens, but also to improve the sensory properties of the product.

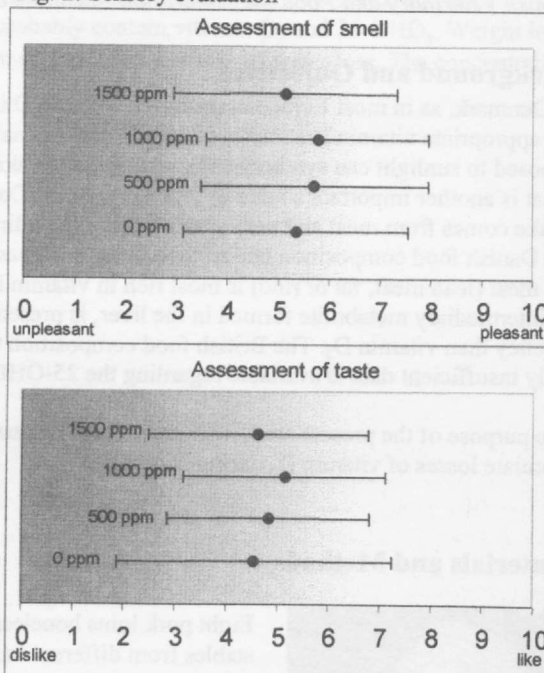
## ABBREVIATIONS USED

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; harman = 1-methyl-9H-pyrido[3,4-b]indole; norharman = 9H-pyrido[3,4-b]indole; ATP = adenosine-tri-phosphat

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Fig. 2: Sensory evaluation



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