

Screening for inhibitors on formation of creatin/ine derived MeIQx - a mutagen/cancerogen Heterocyclic Amine frequently found in heated meat

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Introduction

Thermal processing of meats often enable products to be made edible, make them more appetizing or provide microbiological stability during storage. However, the heating process could also lead to substances with a toxicological relevance on consumers health like Nitrosamines, Polycyclic Aromatic Hydrocarbons (PAHs) or Heterocyclic Amines (HAs). Heterocyclic Amines are produced when Maillard reaction between amino acids, reducing sugars and creatine/ine takes place, and have been shown to be potent mutagens and cancerogens. For this reason the International Agency for Research on Cancer (IARC) has recommended the reduction of human exposure to HAs [1]. One of the most common representatives of HAs is 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx) which can be found in cooked beef, pork, chicken, meat extracts and bacon as well as in cooked fish [2].

Model systems containing the HA precursors creatin/ine, amino acids and sugars are a useful tool to study the inhibiting potential of substances on HA formation because HAs isolated from such model systems were identical with mutagens isolated from cooked meat. The aim of this work was to determine the inhibiting potential of various substances on HA formation in a comparative study performed by statistic experimental design with SAS/QC[®] software. Furthermore, the dose dependent inhibition potency of most effective substances has been studied.

Materials and Methods

Screening experiment and statistical analysis

All experimental designs and statistical analysis were carried out using SAS/QC[®] software and documentation [3]. For the screening experiment a two-level fractional factorial design with blocking was chosen. The factors which affected the content of MeIQx (as the response variable), were the following 8 substances: ascorbic acid; proline; tryptophan; cysteine; N-acetylcysteine; glutathione; tannic acid and Tween 80[®]. For each factor a high level of 0.3 mmol and a low level of 0.03 mmol per mmol creatinine was selected, except for tannic acid and Tween 80[®]. For these substances 0.03/0.003 mmol and 500/50 mg, respectively, were chosen. For the screening experiment we used a 16-run, 8-factor experimental design (see Table 1) with 2 blocks and resolution 4 (main effects not confounded with each other or with two-factor interactions).

Model system

The model system contained the HA precursors creatinine, glycine and glucose in molar concentrations 0.014 mmol, 0.014 mmol and 0.007 mmol, respectively, dissolved in 50 mL water. The 16 samples containing the substances (see Table 1) dissolved in model system solution, were thermally treated at 135 ± 0.1 °C for 1 h in a 170 L laboratory autoclav (Fedegari, Italy).

Dose dependent study

0.05, 0.04, 0.03, 0.02, 0.01, 0.005 and 0.002 mmol per mmol creatinine, of cysteine, N-acetylcysteine and glutathione were weighed in and dissolved with 50 mL of the model system solution. Thermal processing and chemical analysis was identical to the screening experiment.

Sample extraction and HPLC separation

Concentrations of HAs were determined using HPLC, according to the method of Gross and Grüter [4].

Results and Discussion

Figure 1 shows the influence of the different substances on MeIQx content in the screening experiment. All substances in the survey do have a negative effect on MeIQx formation. But this effect is only for cysteine, N-acetylcysteine and glutathione significant ($p < 0.05$). Glutathione has the strongest negative effect (-2.34456) on MeIQx formation. This means that changing the concentration of glutathione from a low level 0.03 to a high level 0.3 mmol/mmol creatinine decreases MeIQx content by 4.68912 ng/mL model system. The 3 substances (cysteine, N-acetylcysteine and glutathione) which show the strongest reduction on MeIQx formation were chosen for the dose depend study. As shown in Figure 2 these substances decrease the content of MeIQx in the model system. As presumed by the screening experiment, the highest reduction could be achieved by the addition of glutathione. With the highest concentration (0.05 mmol of cysteine, N-acetylcysteine and glutathione per mmol creatinine) a MeIQx reduction of 80,4 %, 71,5 % and 91,4 %, respectively, could be achieved. This means adding these substances in only $1/20$ of creatinine content, results in a markable reduction of mutagen formation.

SH-containing substances have previously been reported to inhibit non-enzymatic browning of heated amino acid / glucose mixes [5]. To the best of our knowledge, this is the first study reporting the influence of SH-containing substances on MeIQx formation. The nature of this inhibition is mostly not well understood, and therefore great efforts have been made for a better understanding of the reactions behind the formation.

Conclusions

As shown in the present study, the formation of MeIQx can be significantly reduced by the addition of SH-containing substances like cysteine, N-acetylcysteine or glutathione. Glutathione showed the strongest reduction on MeIQx formation and therefore could be tested as a promising additive to improving the toxicological quality of heat processed meats.



Reference

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Table 1: Weight in [mmol/mmol Creatinine], except Tween

Run No.	Asc.	Cys.	N-Ac.	Glu.	Tan.	Pro.	Try.	Twe.
1	0.03	0.03	0.03	0.3	0.03	0.3	0.3	50 mg
2	0.03	0.03	0.3	0.03	0.03	0.3	0.03	500 mg
3	0.03	0.3	0.03	0.03	0.03	0.03	0.3	500 mg
4	0.03	0.3	0.3	0.3	0.03	0.03	0.03	50 mg
5	0.3	0.03	0.03	0.03	0.003	0.3	0.3	500 mg
6	0.3	0.03	0.3	0.3	0.003	0.3	0.03	50 mg
7	0.3	0.3	0.03	0.3	0.003	0.03	0.3	50 mg
8	0.3	0.3	0.3	0.03	0.003	0.03	0.03	500 mg
9	0.03	0.03	0.03	0.03	0.003	0.03	0.03	50 mg
10	0.03	0.03	0.3	0.3	0.003	0.03	0.3	500 mg
11	0.03	0.3	0.03	0.3	0.003	0.3	0.03	500 mg
12	0.03	0.3	0.3	0.03	0.003	0.3	0.3	50 mg
13	0.3	0.03	0.03	0.3	0.03	0.03	0.03	500 mg
14	0.3	0.03	0.3	0.03	0.03	0.03	0.3	50 mg
15	0.3	0.3	0.03	0.03	0.03	0.3	0.03	50 mg
16	0.3	0.3	0.3	0.3	0.03	0.3	0.3	500 mg

Figure 1: Influence of substances on MeIQx content

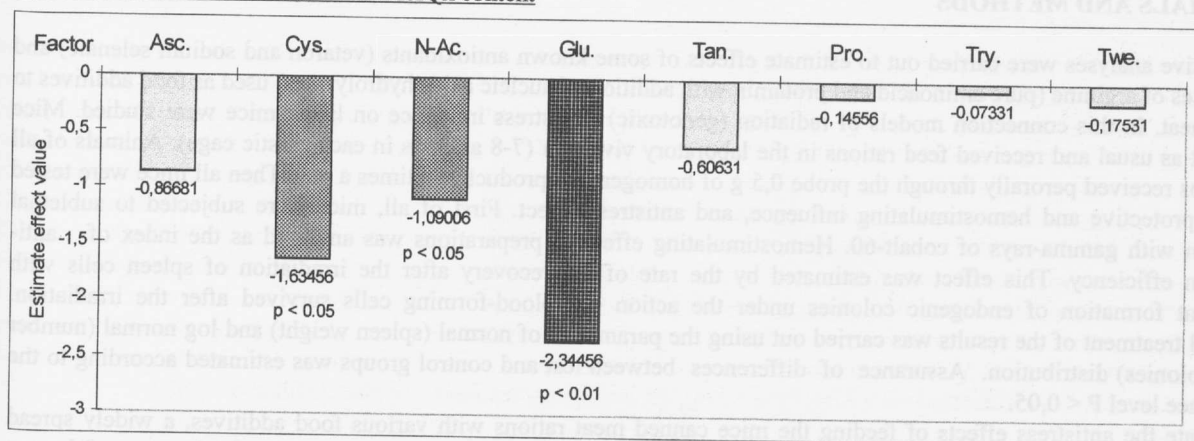


Figure 2: Reduction of MeIQx by SH-containing substances

