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# Effect of Oil Marinades with Garlic, Onion, and Lemon Juice on the Formation of Heterocyclic Aromatic Amines in Fried Beef Patties

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Effects of the application of oil marinades with garlic, onion, and lemon juice on the formation of heterocyclic aromatic amines (HAAs) in fried beef patties were investigated. Two different statistical design models were used. In the screening experiment, a significant inhibition of formation of MeIQx was determined only by addition of garlic ($p < 0.05$). When the amount of garlic was changed from 2 to 20 g/100 g of marinade, the estimated MeIQx content in patties was reduced about 70%. MeIQx (0.38–1.22 ng/g), 4,8-DiMeIQx (n.d.–0.45 ng/g), PhIP (n.d.–0.09 ng/g), norharman (0.76–13.5 ng/g), and harman (2.9–21.5 ng/g) were found in fried patties. The results of two-level and three-level fractional factorial design experiments confirmed the first investigation. They showed a stronger reduction in MeIQx in patties with the addition of increasing amounts of garlic ($p < 0.01$) and onion ($p < 0.05$) in marinades. A higher content of lemon juice in marinades led to only a marginal reduction in MeIQx ($p > 0.05$). The optimum amounts of onion, garlic, and lemon juice that achieved a maximum reduction of HAAs were calculated as 31.2%, 28.6%, and 14.6% in marinade.

**KEYWORDS: Heterocyclic aromatic amines; HAAs; MeIQx; 4,8-DiMeIQx; PhIP; spices; garlic; onion; lemon juice; beef patties; meat products**

## INTRODUCTION

Throughout human history, we have been exposed to potentially mutagenic and carcinogenic compounds including natural occurrence. Epidemiological studies have shown that daily diet could be responsible for the induction of different kinds of cancer. Besides contaminants in the human diet, the great variation in diets and dietary content of food carcinogens may explain the observed worldwide variation in cancer rates (*1*). In the search for possible relationships between diet and cancer, highly mutagenic heterocyclic aromatic amines (HAAs) present in cooked foods were discovered by Japanese scientists around 30 years ago (*2*, *3*). Since their discovery, about 20 HAAs have been identified in cooked foods (*4*, *5*). These substances are found particular in the crust of fried, broiled, and cooked meat and fish. The compounds, with the exception of norharman and harman, are mutagenic in the Ames test and carcinogenic in long-term animal studies on rodents (*6*) and nonhuman primates (*7*, *8*). The International Agency for Research on Cancer (IARC) has classified several HAAs as possible or probable carcinogens and has recommended reducing human exposure to these compounds (*9*). In two European assessment studies of human exposure to HAAs, the total daily mean intake of the main HAAs was determined to be 103 and 160 ng/day, respectively (*10*, *11*). In the U.S. State regulations, as yet the following no significant risk levels (NSRL) were published: 0.41 $\mu$g/day (MeIQx), 0.46 $\mu$g/day (MeIQ), 0.1 $\mu$g/day (Glu-*P*-1), 0.5 $\mu$g/day (Glu-P-2, IQ), 2 $\mu$g/day (A$\alpha$C), 0.6 $\mu$g/day (MeA$\alpha$C), 0.03 $\mu$g/day (Trp-*P*-1), and 0.2 $\mu$g/day (Trp-P-2) (*12*). These levels (NSRL) represent the daily intake level calculated to result in a cancer risk of one excess case of cancer in 100,000 individuals exposed over a 70 year lifetime. In this context, the margin of exposure (MOE) for PhIP shows a very low risk and essentially little concern to human health versus other contaminants (*13*).

The HAAs are usually formed as products of the Maillard reaction. Creatine or creatinine and Maillard products from free amino acids and hexoses such as pyrazines, pyridines, and aldehydes are assumed to form imidazoquinolines, -quinoxalines (IQ-compounds), and -pyridines (*14*). Many factors appear to influence this complex reaction. The important influences of the formation of HAAs are the temperature and the heating time (*15*, *16*), but also the heat transfer to the surface of the product and mass transport of the precursors outward to the crust of meat affect the formation of HAAs.

Some studies have shown that the concentrations of HAAs can be reduced by addition of compounds with an antioxidant potential. Polyphenolic compounds in tea effectively inhibited the formation of HAAs in model systems (*17*, *18*). Some authors demonstrated the inhibitory effect of tart cherry tissue (*19*), carotenoids from tomatoes (*20*), the addition of small amounts of cereal fiber or potato starch (*21*) on the HAAs formation in

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fried ground patties. Also, frying in virgin olive oil reduced the formation of HAAs compared to refined olive oil because the reduction is probably linked to the high content of phenols in virgin olive oil (*22*). Many spices are known for their antioxidant activity. The influence of spices, especially garlic and onion, as inhibitors on the formation of HAAs have been reported (*23–25*). The mutagenicity of cooked beef patties is reduced by the addition of onion to ground beef (*25*). Also studies have shown, that the HAAs content of marinated products is lessened by the application of marinades (*26–28*). The spices onion and garlic contain a lot of organic sulfur compounds. Allicin is the main component of garlic when its cloves are crushed. The main sulfur components are allicin and other thiosulfinates. The latter compounds can be further transformed into diallyl disulfide, diallyl trisulfide, and thioallyl amino acids (*29*). Allicin reverts rapidly to ajoene and dithiins in the presence of edible oils (*30*).

The compound cysteine with a sulfhydryl group can inhibit the Maillard reaction (*31*), which is believed to be a major route in the formation of HAAs. These garlic-related sulfur compounds such as *S*-alkenyl cysteine *S*-oxides, cystine diallyl disulfide, and dipropyl disulfide showed an inhibitive influence on the formation of HAAs (*25*, *32–36*).

The objective of this study was to examine the possibility of reducing the formation of HAAs in beef patties by using several oil marinades with garlic, onion, and lemon juice. The optimum levels of the added marinade components were determined. In addition, the beef patties should have an appetizing color and no overly spicy flavor. The patties were therefore tested for pleasant flavor by a sensory panel.

## MATERIALS AND METHODS

**Materials.** The HAA standards IQ, IQx, MeIQ, MeIQx, 4,8-DiMeIQx, 7,8-DiMeIQx, PhIP, Trp-P-1, Trp-P-2, Glu-P-1, Glu-P-2, AαC, and MeAαC with the following end concentrations in the standard mix (19.7 ng, 22.8 ng, 21.3 ng, 13.6 ng, 12.9 ng, 12.5 ng, 6.5 ng, 5.9 ng, 7.4 ng, 9.4 ng, 5.3 ng and 5.0 ng in 100 μL of methanol) were obtained from Toronto Research Chemicals (Ontario, Canada). All stock solutions of each substance were corrected by means of the extinction coefficient (*37*). Caffeine (internal standard 2.5 μg/mL in ultrapure water and methanol, 1 + 1, v/v), harman, and norharman (standard mix 4.9 ng and 4.1 ng in 100 μL of methanol) were obtained from Sigma-Aldrich (Taufkirchen, Germany). The following materials were used: diatomaceous earth isolute (Separtis, Germany), propylsulfonic acid (PRS) (100 mg), and C 18 Bond Elut cartridges 500 mg and 100 mg (Varian Inc., USA). All other chemicals were analytical grade or gradient grade for HLPC (Merck, Germany).

**Preparation of beef patties and frying conditions.** *Experiments A and B.* Pure beef (MEGA, Stuttgart, Germany), roughly desinewed and defatted, was coarsely minced through a 3 mm plate. Salt (1.2 g/100 g) was added to the minced beef separately and was mixed with a blender. An 80 ± 1 g portion of this material were formed into beef patties with a special mold for beef patties (16 mm thick × 85 mm diameter, 12.3% fat). The patties were fried at 230 °C for 5 min with a double contact grill (Nevada, Neumärker, Bad Bramstedt, Germany). The marinated beef patties were laid between two sheets of aluminum foil. Four patties were fried together with the double contact grill. For each batch, eight patties were fried on both sides simultaneously to a core temperature of 72 °C and to a surface temperature <200 °C at the end of the frying process. Surface and core temperature were monitored during the frying process with a temperature data logger (Almemo 8990/8, Ahlborn, Holzkirchen, Germany).

*Experiment C.* Prepared deep frozen beef patties 62.4 g (Salomon Hitburger, Grossostheim, Germany) without spices (6 mm thick × 100 mm diameter, 21.8% fat) were used. The optimal heating time was 1:50 min for these patties. The core temperature was measured only at the end of the frying process. The others frying conditions were along the lines of experiment B.

**Table 1.** Experiment A: Marinades with Different Concentrations of Lemon Juice, Onion, and Garlic in Sunflower Oil with 10% Olive Oil (A) or 100% Olive Oil (B)

| marinades with edible oil | onion (g/100 g) | garlic (g/100 g) | lemon juice (g/100 g) | oil |
|---|---|---|---|---|
| marinade 1 | 5 | 20 | 20 | A |
| marinade 2 | 5 | 2 | 2 | A |
| marinade 3 | 5 | 2 | 20 | B |
| marinade 4 | 50 | 2 | 20 | A |
| marinade 5 | 50 | 20 | 2 | A |
| marinade 6 | 5 | 20 | 2 | B |
| marinade 7 | 50 | 2 | 2 | B |
| marinade 8 | 50 | 20 | 20 | B |

**Table 2.** Experiment B: Marinades with Different Concentrations of Lemon Juice, Onion, and Garlic in Olive Oil (g/100 g) and the Determined Mean Concentrations of MeIQx and 4,8-DiMeIQx ($n = 4$)

| run no. | block | onion (g/100 g) | garlic (g/100 g) | lemon juice (g/100 g) | MeIQx (ng/g) | 4,8-DiMeIQx (ng/g) |
|---|---|---|---|---|---|---|
| 1 | 1 | 10 | 10 | 5 | 0.22 | 0.11 |
| 2[a] | 1 | 20 | 20 | 10 | 0.12 | 0.04 |
| 3[a] | 1 | 20 | 20 | 10 | 0.17 | 0.05 |
| 4 | 1 | 10 | 30 | 15 | 0.12 | 0.05 |
| 5 | 1 | 30 | 10 | 15 | 0.18 | 0.07 |
| 6 | 1 | 30 | 30 | 5 | 0.06 | 0.04 |
| 7[a] | 2 | 20 | 20 | 10 | 0.19 | 0.09 |
| 8[a] | 2 | 20 | 20 | 10 | 0.24 | 0.09 |
| 9 | 2 | 30 | 10 | 5 | 0.27 | 0.14 |
| 10 | 2 | 30 | 30 | 15 | 0.05 | 0.02 |
| 11 | 2 | 10 | 30 | 5 | 0.26 | 0.08 |
| 12 | 2 | 10 | 10 | 15 | 0.38 | 0.13 |
| 13 | 3 | 36.82 | 20 | 10 | 0.13 | 0.07 |
| 14[a] | 3 | 20 | 20 | 10 | 0.16 | 0.06 |
| 15 | 3 | 3.18 | 20 | 10 | 0.3 | 0.15 |
| 16[a] | 3 | 20 | 20 | 10 | 0.17 | 0.08 |
| 17 | 3 | 20 | 20 | 1.59 | 0.31 | 0.18 |
| 18 | 3 | 20 | 3.18 | 10 | 0.43 | 0.18 |
| 19 | 3 | 20 | 20 | 18.41 | 0.13 | 0.06 |
| 20 | 3 | 20 | 36.82 | 10 | 0.18 | 0.03 |

[a] Marinades with the same composition.

**Preparation of Marinades.** All ingredients of the marinades were obtained from a local grocery store. After the skins were removed, the fresh cloves of garlic and onions were finely minced with a household cutter. The lemon juice was handmade freshly squeezed from lemons. The compositions of marinades were made according to designs of experiments. The marinades were repeatedly shaken. After 24 h, the marinades were shaken once more and were used for coating of the beef patties (1.5 g per side).

**Statistical Analysis.** All experimental designs and statistical analyses were carried out using SAS/QC software 6.12 (SAS Institute Inc., Cary, NC).

*1. Experiment A (Screening Test).* The patties were coated with eight different marinades each with two concentrations of lemon juice, minced cloves of garlic, onions, and virgin olive oil in sunflower oil, corresponding to a statistical two-level fractional factorial design model as a screening experiment (eight runs, resolution 4, four factors) (**Table 1**).

*2. Experiment B.* This is a response surface model for the response variable MeIQx (**Table 2**). The variable MeIQx is a function relating to three factors, the concentrations of onion, garlic, and lemon juice. Therefore, a Box-Wilson design with uniform precision was selected. Uniform precision means that the variance of the predicted response is the same for all points near the center of design. This design contains 20 runs with 3 blocks with 6 center points. The blocking is necessary because the analytical determination cannot be performed on the same day. This response surface model with the ridge analysis allowed

**Table 3.** Experiment C: Marinades with Different Concentrations of Onion and Garlic inOlive Oil (g/100 g)

| run no. | block | onion (g/100 g) | garlic (g/100 g) |
|---|---|---|---|
| 1 | 1 | 10 | 5 |
| 2 | 1 | 40 | 25 |
| 3[a] | 1 | 25 | 15 |
| 4[a] | 1 | 25 | 15 |
| 5[a] | 1 | 25 | 15 |
| 6 | 1 | 10 | 25 |
| 7 | 1 | 40 | 5 |
| 8 | 2 | 25 | 0.86 |
| 9 | 2 | 46.21 | 15 |
| 10 | 2 | 25 | 29.14 |
| 11[a] | 2 | 25 | 15 |
| 12[a] | 2 | 25 | 15 |
| 13 | 2 | 3.79 | 15 |
| 14[a] | 2 | 25 | 15 |

[a] Marinades with the same composition.

**Table 4.** HPLC Gradient Program at a Flow Rate of 1 mL/min and 25 °C

| time (min) | eluent A (%) | eluent B (%) | eluent C (%) |
|---|---|---|---|
| 0–10 | 82–75 | 10 | 8–15 |
| 10–20 | 0 | 85–75 | 15–25 |
| 20–29 | 0–5 | 75–55 | 25–45 |
| 29–30 | 5–57 | 55–35 | 45–8 |
| 30–33 | 57–82 | 35–10 | 8 |
| 33–36 | 82–15 | 10 | 8–75 |
| 36–40 | 82–15 | 10 | 75 |
| 40–42 | 15–82 | 10 | 75–8 |
| 42–52 | 82 | 10 | 8 |

determination of the optimum and minimum levels for the concentration of MeIQx according to all factors. In this case, the goal is to minimize the concentration of HAA compounds.

*3. Experiment C.* This was performed along the lines of experiment B, but only the two significant factors garlic and onion of experiment B were used. This design contains 14 runs with two blocks with six center points (**Table 3**). For the removal of the factor lemon juice, a regression analysis was used with backward selection of nonsignificant factors ($p = 0.1$).

**Determination of HAAs.** The method included the polar and nonpolar HAAs. The method of HPLC analysis with some modifications was based on the described method (*38*, *39*).

Extracts were taken from homogenized fried beef patties by blending 30 g with 90 g of 1 M sodium hydroxide in an Ultra Turrax (Janke & Kunkel, Staufen, Germany) for 4 min with high speed. The homogenate was divided into four equal 20 g portions. Two portions were spiked with 100 $\mu$L of the standard mixture. Each of the four portions was mixed with 15 g of diatomaceous earth and filled in blank columns. The HAAs were extracted with a dichloromethane solution with 5% toluene (100 mL) and absorbed onto a coupled PRS cartridge, which was preconditioned with a dichloromethane/toluene mixture (4 mL). The PRS cartridge was dried by a low-level nitrogen flow. After the sample was washed with 6 mL of 0.1 M hydrochloric acid (HCl), the nonpolar HAAs were eluted with a mixture of 15 mL of 0.1 M HCl/methanol (2:3, v/v). The cartridge was washed with ultrapure water (6 mL). The combined eluents with washing solution was rendered alkaline with 0.5 mL of ammonia (25%). The nonpolar HAAs were adsorbed onto a coupled preconditioned C18 cartridge (500 mg, 2 mL of methanol, and 2 mL of ultrapure water). After the sample was washed with ultrapure water (2 mL) and dried, the nonpolar HAAs were eluted with 1.2 mL of methanol/ammonia (25%) mixture (90:10, v/v)l. After drying by a nitrogen flow to dryness in an evaporator at 40 °C (Barkey, Leopoldshöhe, Germany), the eluate was redissolved in 100 $\mu$L of caffeine solution (internal standard). The polar HAAs were eluted from the PRS cartridge with 20 mL of ammonia acetate (0.5 M, pH 8) and adsorbed onto a coupled preconditioned C18 cartridge (100 mg, 1 mL of methanol, and 1 mL of ultrapure water). After the sample was washed with ultrapure water (2 mL) and dried, the polar HAAs were eluted (with 0.8 mL of methanol/ammonia (25%) mixture (90:10, v/v) and redissolved in 100 $\mu$L of internal standard after drying.

**Table 5.** Recoveries of Found HAAs in the Fried Beef Patties after Extraction and Cleanup Steps (Experiment A)

| recoveries ($n = 8$) | mean (%) | std dev (%) | rel variation coeff (%) |
|---|---|---|---|
| MeIQx | 62.2 | 5.9 | 9.4 |
| 4,8-DiMeIQx | 92.4 | 9.5 | 10.3 |
| PhIP | 51.7 | 14.7 | 24.2 |
| norharman | 65.0 | 11.2 | 17.3 |
| harman | 87.1 | 14.2 | 16.3 |

A previously described HPLC method (*38*) was modified and used as follows. The HPLC equipment used for this analysis was a Gynkotek HPLC system (Gynkotek, Germering, Germany), Pump M480, autosampler Gina 50, degasser (DG 1310 S), equipped with a fluorescence detector (RF 1002), diode array detector (UVD 320), and the Gynkosoft chromatography data system (version 5.50). The HAAs were analyzed using the column TSK-gel ODS-80Tm 250–4.6, (Tosoh-Biosep, Stuttgart, Germany) and the guard column Supelguard$^{TM}$ LC-18-DB (Supelco, USA). The mobile phase consisted of eluent A, 0.01 M triethylamine phosphate buffer (pH 3.2), eluent B, 0.01 M triethylamine phosphate buffer (pH 3.6), and eluent C, acetonitrile. The gradient program is shown in **Table 4**. The UV detection was performed at 258 nm and 3D field for spectra plots (200–360 nm). The adjustment of fluorescence detector ($\lambda$ ex/em) was from 0 min (360/450 nm), 14 min (300/440 nm), 22 min (265/410 nm), 24 min (305/390 nm), 25.5 min (265/ 410 nm), and 28 min (335/410 nm).

The peaks of HAAs in samples, including norharman and harman, were identified by comparing the retention times and UV spectra with standards. The quantification was carried out with an external calibration (norharman, harman) or standard addition (MeIQx, 4,8-DiMeIQx, PhIP) with one standard concentration. The results were corrected for incomplete recovery.

**Determination of Creatine/Creatinine.** Creatine/creatinine were enzymatically determined according to test instructions from Roche diagnostics GmbH (*40*). All chemicals were obtained from Roche diagnostics GmbH (Roche diagnostics GmbH, Mannheim, Germany).

**Sensory Tests.** The task of the sensory panelists ($n = 23$) was to evaluate the patties of the first experiment for odor, flavor, and color using a 10 score scale. For odor and flavor, the panelists could be chosen on a scale between like very much (10) and do not like at all (0), and for color the scale was very light (0), optimal (5), and very dark (10). The testers, which all like garlic, were trained with patties fried with different heating times and sensory ranking tests (data not shown). The evaluation of the sensory results was used a variance analysis (Tukey test).

## RESULTS AND DISCUSSION

The recoveries of the found HAAs in the investigated samples (experiment A) after the extraction and the cleanup steps are shown in **Table 5**. The HPLC separation of the polar fraction with a spiked and unspiked sample and a standard mixture is demonstrated in **Figure 1**. The contents of HAAs were corrected for incomplete recovery by using the standard addition. The recovery rates are comparable with other studies (*41–43*). The average difference between duplicate determinations was 0.08 ng/g for MeIQx and 0.04 ng/g for 4,8-DiMeIQx (experiment A, $n = 8$). The quantification limit of the method is 0.02 ng/g for all HAAs, because the distinct identification was carried out with the UV spectra.

The self-made beef patties used were selected because the product had a standardized form, the same weight and raw material. The frozen prepared patties had a higher fat content (21.8%) than the other raw material (10.3%) in the first and second experiment (A and B). Also the total creatine concentrations based on dry matter in the raw meat were

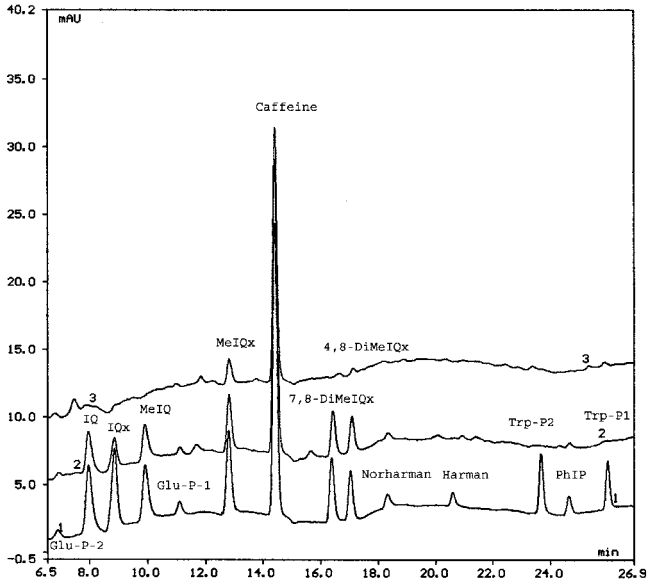


**Figure 1.** HPLC/UV chromatogram (258 nm) of the polar fraction of a spiked (1) and unspiked (2) beef patties (experiment A, marinade 3) as well as a standard mixture of HAAs (3).

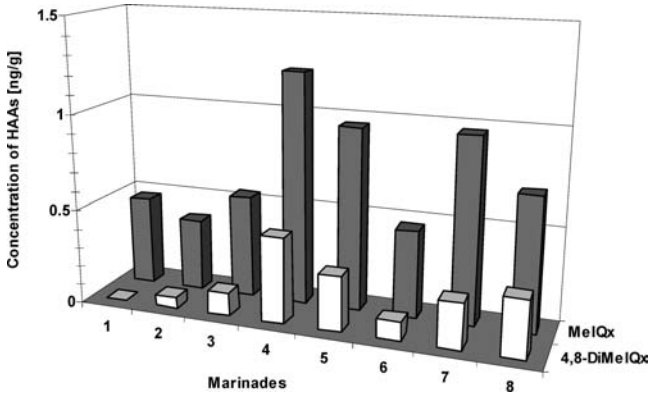


**Figure 2.** Concentrations of MeIQx and 4,8-DiMeIQx in fried patties marinated with eightdifferent marinades (experiment A).

highly variable with the values at 1.2 g/100 g (experiment A and B) and 0.8 g/100 g (experiment C). The contents of total creatine in raw meat are comparable to other published studies (*44–46*) and were measured to ensure the amount and quality of the meat products (*47*), because the content of creatine is higher in lean meat than in meat with more connective and fat tissue. The concentrations of connective tissue were determined to be 2.5 g/100 g in the self-made beef patties and 3.5 g/100 g in the prepared deep frozen beef patties.

As a result of preliminary studies, it was decided to marinate and fry the self-made beef patties with the different oil marinades for 5 min at a measured surface temperature of 190−200 °C, which gave well-done medium brown products with good organoleptic properties.

The different composition of spices used in oil marinades can affect the formation of HAAs. Four HAAs, MeIQx, 4,8-DiMeIQx (**Figure 2**), norharman (0.76–13.5 ng/g), and harman (2.9–21.5 ng/g), were found in the patties, while Glu-P-1, Glu-P-2, IQ, MeIQ, IQx, 7,8-DiMeIQx, Trp-P-1, Trp-P-2, PhIP, AαC, and MeAαC were not detected. In **Figure 2**, the four samples with the highest MeIQx concentrations all contained the highest concentration of onion. Onions contain much sugar, and earlier studies have shown that at some

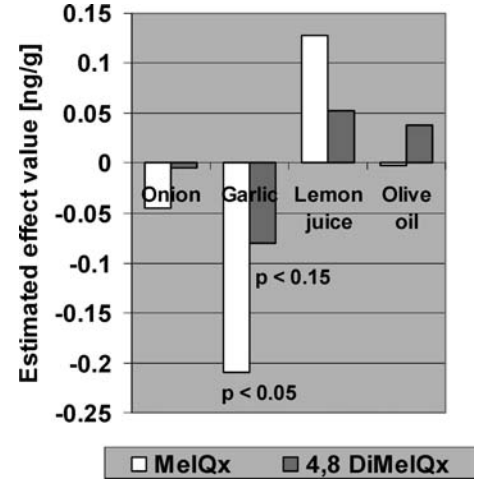


**Figure 3.** Main effects on content of HAAs in marinated and fried patties (experiment A).

concentrations sugar can enhance the formation of HAAs (*48*). In the present study, the found mean difference of the batches between the content of HAAs in marinated and fried patties was considerable from the lowest (0.38/0.02 ng/g) to the highest concentration (1.22/0.45 ng/g) for MeIQx and 4,8-DiMeIQx, respectively. An inhibition of the MeIQx formation in the patties was determined to be significant only when garlic was added to the marinades ($p < 0.05$). The concentration of 4,8-DiMeIQx was also reduced by garlic, but the effect was not significant ($p < 0.15$) (**Figure 3**). If the amount of garlic was changed one order of magnitude from 2 to 20 g/100 g in the marinades, the estimated MeIQx content in the patties would decreased by 0.42 ng/g in this test. In **Figure 3**, the strongest effect was calculated for the garlic factor with the estimated effect value of −0.21 ng/g for MeIQx, because this value is computed to the center amount of the model. The concentrations of norharman (0.9–13.5 ng/g) and harman (2.9–21.5 ng/g) increased with higher concentrations of garlic, onion, and lemon juice in marinades. The highest values for both $\beta$-carbolines were measured for the marinade with the highest amount of garlic, onion, and lemon juice (marinade 8). However, the effect was not significant for any factor ($p > 0.15$). The $\beta$-carbolines norharman and harman do not possess any mutagenic activity but become mutagenic with nonmutagenic aromatic amines and enhance the mutagenic potential of Trp-*P*-1 and Trp-P-2 (*49*). Also these compounds were associated with neurological diseases (*50*).

The addition of lemon juice showed a slight increased formation of MeIQx and 4,8-DiMeIQx, but all these effects were not significant ($p > 0.15$). The different content of olive oil in sunflower oil did not significantly influence the concentrations of HAAs. The basic oil of the marinade, sunflower or olive oil, had no significant effect on HAA concentrations in this test. Therefore in experiments B and C, the marinades were prepared with olive oil like the traditional marinades of Mediterranean countries. Other authors, however, described HAA reducing effects of virgin olive oil; this effect is probably due to the content of phenols in virgin olive oil (*22*).

The concentrations of MeIQx and 4,8-DiMeIQx are shown in **Table 2**. The statistical results of the response surface analysis (experiment B), with only three factors, garlic, onion, and lemon juice, and their interactions, confirmed the first investigation. They showed a stronger reduction of MeIQx

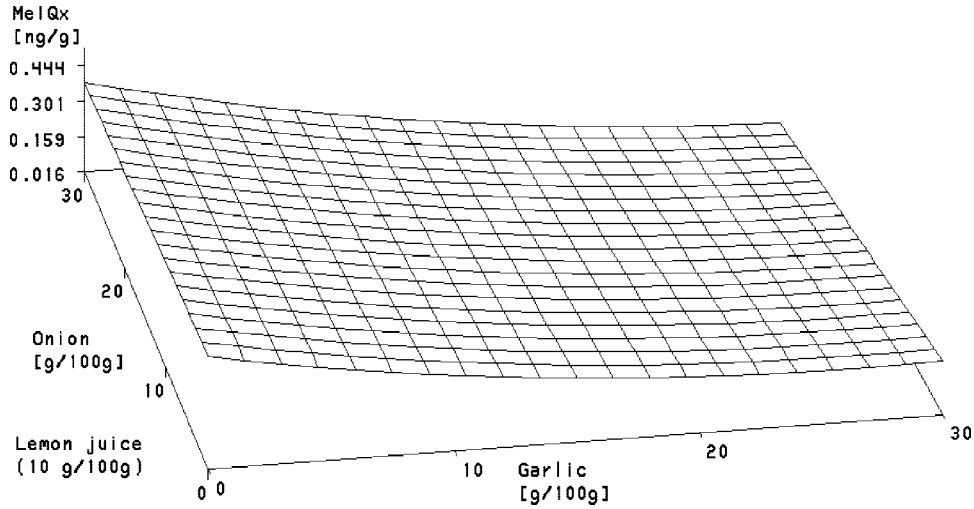


**Figure 4.** Effect of garlic and onion on the concentration of MeIQx (lemon juice was constant at 10 g/100 g) (experiment B). Following fitted response surface: ($C_{MeIQx} = 0.398 + 0.00035C_{onion} - 0.0119C_{garlic} + 0.009C_{lemon} + 0.00001C_{onion}^2 - 0.00015C_{garlic}C_{onion} + 0.00033C_{garlic}^2 - 0.0003C_{lemon}C_{onion} - 0.00055C_{lemon}C_{garlic} + 0.00012C_{lemon}^2$).

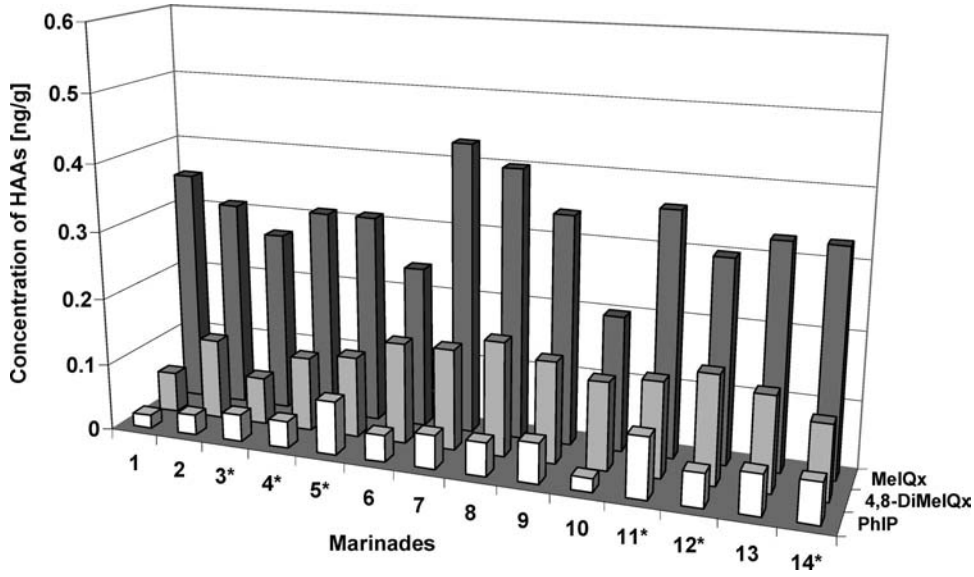


**Figure 5.** Concentration of HAAs in fried patties marinated with different marinades. Following fitted response surface (experiment C): ($C_{MeIQx} = 0.393 - 0.00088C_{onion} - 0.00412C_{garlic} + 0.00005C_{onion}^2 - 0.00002C_{garlic}C_{onion} - 0.00006C_{garlic}^2$). Asterisks indicate marinades with the same composition.

in the patties with the addition of increasing amounts of garlic ($p < 0.01$) and onion ($p < 0.05$) to the marinades. A higher content of lemon juice in the marinades led to less reduction in MeIQx, and the concentration of MeIQx increased significantly without garlic and high amounts of lemon juice. But the addition of lemon juice in combination with the other ingredients to the marinades showed neither significant positive nor negative effects. The maximum level for MeIQx resulted from the combination of onion, garlic, and lemon juice in ratio of 17.2 to 3.5 and to 11 g/100 g. To minimize the MeIQx content, the optimum levels of onion, garlic, and lemon juice were calculated at 31.2, 28.6, and 14.6 g/100 g in the marinade by using the statistical ridge analysis (**Figure 4**). Also the statistical test used did not indicate any significant interactions with the other factors.

Therefore in experiment C, the olive oil marinade contained only onion and garlic in different concentrations. However, the ingredient garlic had significant effects on the MeIQx content ($p < 0.001$) and the interaction of garlic and onion ($p < 0.05$). In comparison to the equation of fitted response surface for MeIQx, the theoretical single addition of onion had no significant calculated effect in this experiment (**Figure 5**). To minimize the MeIQx content, the optimum amounts of onion and garlic were calculated at 21.8% and 28.9% in the marinade with olive oil; the MeIQx level was computed to 0.2 ng/g. The HAA concentrations and the calculated fitted response surface are indicated in **Figure 5**. The concentration of MeIQx can be reduced about 50% from the maximum (0.41 ng/g MeIQx) to the minimum level (0.2 ng/g MeIQx, 38.5/4.1 g/100 g onion/garlic). The concentrations of PhIP were determined to be between 0.02 and 0.09 ng/g in the beef patties fried with the 14 different marinades (**Figure 5**). The PhIP concentrations of the most batches were close to the determination limit of the analytical method. For this reason, the evaluation of this statistical design experiment is difficult. Therefore, no significant effects were calculated in all probability for the compounds PhIP and 4,8-DiMeIQx in this design experiment. In all these experiments, the main reducing effect on the content of MeIQx was shown by application of the added minced garlic in the marinades. The optimum amount of garlic for the marinades was defined at

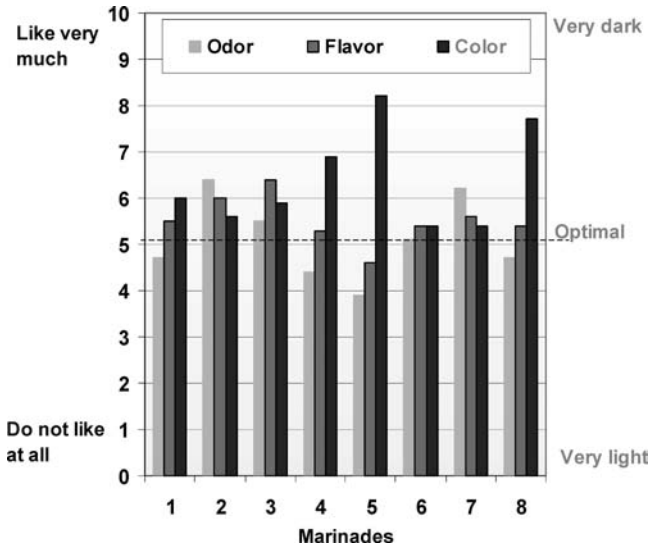


**Figure 6.** Results of the sensory test with patties marinated in eight various marinades (experiment A).

28–29 g per 100 g of oil marinade in both these design experiments (response surface models).

Garlic and onion have a lot of compounds with sulfhydryl groups which were mainly formed by an enzymatic reaction after mincing. An inhibiting effect of different sulfur compounds such as cysteine, acetylcysteine, and glutathione on MeIQx formation in a meat matrix based model system or diallyl disulfide has been reported (*32, 35, 36*). Also these compounds and deoxyalliin inhibited the mutagenic potential in the Ames test and in part the brown color formation in the model system (*36*). In the Maillard reaction, sulfur amino acids may also participate in meat flavor. Alliin and deoxyalliin reacted with glucose, and these isolates possessed a roasted-meat-like flavor (*51*). Some authors have shown that the effect of marinating with Asian marinades (terriyaki sauce and tumeric sauce) reduced the content of PhIP and MeIQx in cooked meat, as well as mutagenicity as measured by the Ames/Salmonella test (*26*). These marinades, which contain fresh minced garlic or garlic powder, are commonly used by Japanese or Asian Indians to marinate fish and meat. A reduction in the formation of HAAs, and especially the mutagenic activity, was also described for grilled chicken samples with commercial marinades (*28*).

Similar results were found in other investigations in which sauces were used to marinate chicken or fish before frying (*27, 52*). These products contained ingredients such as garlic, olive oil, brown sugar, or lemon juices, in addition to other compounds. After the food was grilled, the marinated products had less PhIP, but more MeIQx. In domestically prepared chicken and fish from Singapore Chinese households, a partial reduction was only demonstrated in the formation of PhIP after application of marinades before grilling (*52*).

The direct addition of garlic and onion to the ground beef of the patties frequently resulted in overspiced products. The application of marinades had the advantage that the beef patties got a positive evaluation for odor and flavor from the sensory testers (**Figure 6**). Only patties with marinades of batchs 5 and 8 (experiment A) were significantly darker than other batches ($p < 0.05$). The marinades with the highest content of onions resulted in the darkest color. Especially the sugar content of onion as well as garlic could influence the color as a consequence of the Maillard reaction. The evaluation of the odor and flavor showed no significant difference between the batches.

To sum up this study, garlic and, to a minor degree, onion showed inhibitory effects on the formation of MeIQx and 4,8-DiMeIQx. Marinating with the different marinades had partially a slight promoting effect of the content of comutagenic $\beta$-carbolines norharman and harman in some cases. The compound PhIP was found only in concentrations near the detection limit of the analytical method, so that no statistical evaluation was possible.

Nevertheless, the present data clearly confirm that MeIQx and 4,8-DiMeIQx formation is affected by marinating with garlic and onion. This marinating preparation of meat is a traditional treatment before grilling over burning charcoal or wood in Mediterranean countries. For evaluation the daily human exposure of HAAs in epidemiological studies, the use of marinades should also be considered along with the cooking method and doneness of the meat products.

## ABBREVIATIONS USED

HAAs, heterocyclic aromatic amines; IQ, 2-amino-3-methylimidazo[4,5-*f*]quinoline; IQx, 2-amino-3-methylimidazo[4,5-*f*]quinoxaline; MeIQ, 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline; MeIQx, 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; 4,8-DiMeIQx, 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline; 7,8-DiMeIQx, 2-amino-3,7,8-trimethylimidazo[4,5-*f*]quinoxaline; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; Trp-P-1, 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole; Trp-P-2, 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole; Glu-P-1, 2-amino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazole; Glu-P-2, 2-aminodipyrido[1,2-*a*:3′,2′-*d*]imidazole; AαC, 2-amino-9*H*-pyrido[2,3-*b*]indole; MeAαC, 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole; harman, 1-methyl-9*H*-pyrido[3,4-*b*]indole; norharman, 9*H*-pyrido[3,4-*b*]indole; n.d., not detected; em, emission; ex, excitation; *C*, concentration.

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