EFFECT OF MARINATING PRETREATMENT WITH SPICES ON THE FORMATION OF MUTAGENIC/CARCINOGENIC HETEROCYCLIC AMINES IN FRIED BEEF PATTIES

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Background:
Epidemiological studies have shown that the daily diet could be responsible for the initiation of different kinds of cancer. The great variation in human diets and dietary content of food carcinogens may explain the observed variation in cancer rates worldwide. The Heterocyclic Amines (HA) are especially found in the crust of fried, broiled and cooked meat and fish. The HA are carcinogenic in long-term animal studies on rodents and monkeys (ADAMSON et al., 1990). The International Agency on Cancer Research has classified several HA as possible carcinogens and has recommended reducing human exposure to these compounds (IARC, 1993). The HA 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 imidazo-quinolines, -quinoxaline (IQ-compounds) and -pyridines. Many factors appear to influence this complex reaction. Some studies have shown the influence of antioxidants (JOHANSSON et al., 1996) and spices, especially garlic and onion, on the formation of HA (MURKOVIC et al., 1998, GIBIS et al., 1999). The spices onion and garlic contain a lot of compounds with sulfhydryl groups. These reducing compounds such as cysteine can inhibit the Maillard reaction which is believed to be a major route in the formation of HA (FRIEDMAN, 1996).

Objectives:
The aim of this study was to examine the possibility of reducing the formation of HA in beef patties by using several oil marinades with garlic, onion and lemon juice. The beef patties were tested to a pleasant flavour by a sensory panel.

Materials and Methods:
Preparation of beef patties: Pure beef, roughly desinewed and defatted, was coarsely minced through a 3 mm plate. 1.2 % salt was added to the minced beef separately and was mixed with a blender. 80 g ± 1 g of the material were formed into beef patties with a special mold for hamburgers.
Statistical analysis: All experimental designs and statistical analysis were carried out using SAS/QC software (SAS, 1994).
1. Experiment: The patties were coated with 8 different marinades with sun flower oil and several concentrations of olive oil, lemon juice, minced garlic and onion corresponding to a statistical two-level fractional factorial design model as a screening experiment (8 runs, resolution 4, 4 factors) (Tab. 1). The patties were fried at 230°C for 5 min with a double contact grill (Nevada, Neumärker, D).

2. Experiment: This is a response surface model for the response variable MeIQx (Tab.2). The variable MeIQx is a function relating to 3 factors the concentration 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 centre of design. This design contains 20 runs with 3 blocks with 6 centre points.

Tab.1: Experiment 1: Marinades with different concentration of lemon juice, onion and garlic in sun flower oil with 10 % olive oil (A) or 100 % olive oil (B)

Marinades with edible oil	Onion [g/100g]	Garlic [g/100g]	Lemon juice [g/100g]	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

Determination of HA: The method included the polar and apolar HA. The method of HPLC analysis with some modifications was based on the method described by GROSS and GRÜTER (1992). The peaks of HA, also Norharman and Harman, in samples were identified by comparing the retention times and UV-spectra with standards. The quantification was carried out with an external calibration.
Sensory tests: The task of the sensory panellists was to evaluate the patties of the first experiment for odour, flavour and colour using a 10 score scale.

Tab. 2: Marinades with different concentration of lemon juice, onion and garlic in olive oil [g/100g]

Marinades Run No.	Block	Onion [g/100 g]	Garlic [g/100 g]	Lemon juice [g/100 g]
1	1	10	10	5
2	1	20	20	10
3	1	20	20	10
4	1	10	30	15
5	1	30	10	15
6	1	30	30	5
7	2	20	20	10
8	2	20	20	10
9	2	30	10	5
10	2	30	30	15
11	2	10	30	5
12	2	10	10	15
13	3	36.82	20	10
14	3	20	20	10
15	3	3.18	20	10
16	3	20	20	10
17	3	20	20	1.59
18	3	20	3.18	10
19	3	20	20	18.41
20	3	20	36.82	10

Results and Discussions:

MeIQx, 4,8-DiMeIQx (2-amino-3,4,8-trimethylimidazo[4,5-f] quinoxaline) (Fig. 1), Norharman (0.76-13.5 ng/g) and Harman (2.9-21.5 ng/g) were found in the patties. An inhibition of the formation of MeIQx in the patties was determined to be significant only by the addition of garlic to the marinades ($p< 0.05$). The concentration of 4,8-DiMeIQx was also reduced by garlic, but the effect was not significant ($p < 0.15$) (Fig. 2). If the amount of garlic was changed from 2 to 20 g/100g in the marinades, the estimated MeIQx content in the patties was decreased by 0.4 ng/g in this test (Fig. 2). The concentrations of Norharman and Harman increased with higher concentrations of garlic, onion and lemon juice. The addition of lemon juice showed an increased formation of MeIQx and 4,8-DiMeIQx, but all these effects were not significant. The different content of olive oil did not influence the concentrations of HA.

The results of the response surface analysis with only 3 factors garlic, onion, lemon juice and their interactions confirmed the first investigation. They showed a stronger reduction in MeIQx 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 without garlic and high amounts of lemon juice, but this effect was not significant. To minimise the MeIQx content, the optimal levels of onion, garlic and lemon juice were calculated at 31.2 %, 28.6 % and 14.6 % in the marinade by using the statistical ridge analysis (Fig. 3).

Garlic and onion have a lot of compounds with sulfhydryl groups which were mainly formed by an enzymatic reaction after mincing. These substances have a reducing effect on the formation of MeIQx. An inhibiting effect of different organo sulphur compounds such as cysteine, acetylcysteine and glutathione on MeIQx formation in a meat matrix based model system has been reported (SCHOCH et al., 1998).The direct addition of garlic and onion to the patties resulted in overspiced products. The application of marinades have the advantage that the beef patties got a positive evaluation for odour and flavour from the sensory testers (Fig. 4). Only patties with marinade 5 and 8 are significantly darker than the others ($p< 0.05$). The marinating of meat is a traditional treatment before grilling in Mediterranean countries.

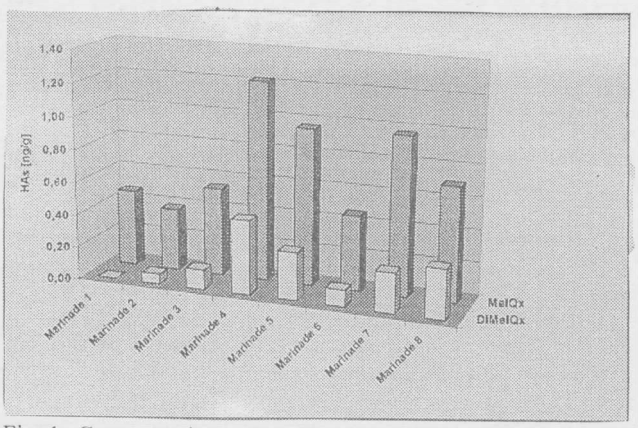

Fig. 1: Concentrations of MeIQx and 4,8-DiMeIQx in fried patties coated with 8 different marinades (experiment 1)

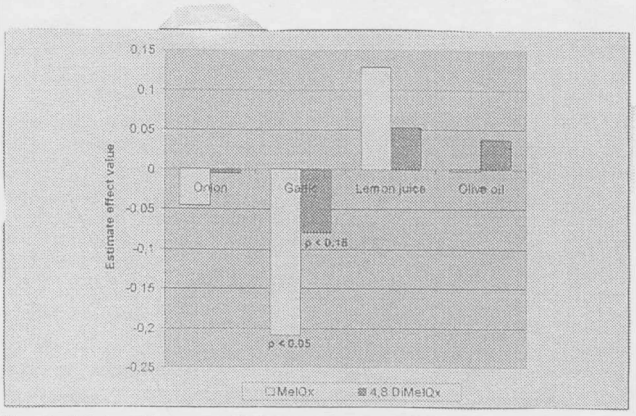

Fig. 2: Main effects on HA content in marinated patties

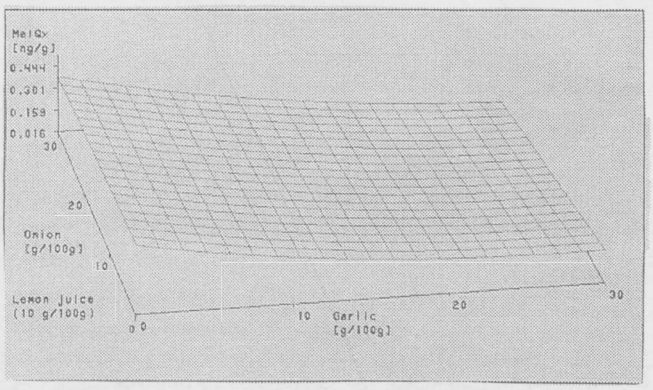

Fig. 3: Effect of garlic and onion on the concentration of MeIQx (lemon juice: constant 10 g/100g)

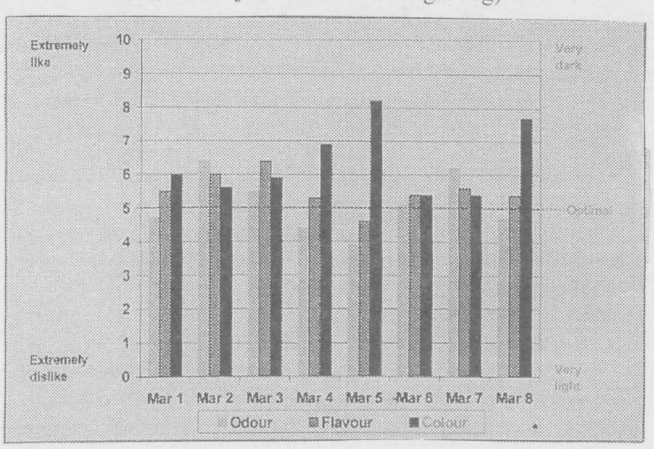

Fig. 4: Results of the sensory test with patties coating with 8 various marinades (experiment 1)

Conclusions:

The results demonstrated that the addition of onion and garlic to the oil marinades have the effect of reducing the formation of carcinogenic MeIQx and 4,8-DiMeIQx in the fried beef patties. These spices are normally used in the marinating of meat before grilling in Mediterranean countries.

References:

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