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Inhibitory effect of marinades with hibiscus extract on formation of heterocyclic aromatic amines and sensory quality of fried beef patties

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ABSTRACT

Heterocyclic aromatic amines (HAA) are carcinogenic compounds found in the crust of fried meat. The objective was to examine the possibility of inhibiting HAA formation in fried beef patties by using marinades with different concentrations of hibiscus extract (*Hibiscus sabdariffa*) (0.2, 0.4, 0.6, 0.8 g/100 g). After frying, patties were analyzed for 15 different HAA by HPLC-analysis. Four HAA MeIQx (0.3–0.6 ng/g), PhIP (0.02–0.06 ng/g), co-mutagenic norharmane (0.4–0.7 ng/g), and harmane (0.8–1.1 ng/g) were found at low levels. The concentration of MeIQx was reduced by about 50% and 40% by applying marinades containing the highest amount of extract compared to sunflower oil and control marinade, respectively. The antioxidant capacity (TEAC-Assay/Folin–Ciocalteu-Assay) was determined as 0.9, 1.7, 2.6 and 3.5 μmol Trolox antioxidant equivalents and total phenolic compounds were 49, 97, 146 and 195 μg/g marinade. In sensory ranking tests, marinated and fried patties were not significantly different ($p > 0.05$) to control samples.

1. Introduction

Several epidemiological studies have shown a relationship between a high daily intake of meat and increased risk of carcinogenesis. A number of studies have also reported that the preparation method and/or degree of cooking (well done, medium, rare, etc.) could be contributing factors to this increased risk (Rohrmann & Becker, 2002; Rohrmann, Hermann, & Linseisen, 2009). A possible relationship between the intake of HAA and the incidence of different cancers such as colon, breast, stomach, and pancreas has been demonstrated (Butler et al., 2003; Nowell et al., 2002; Sinha, 2002). A total daily mean intake of the major HAA was reported to be 103 and 160 ng/day, respectively in two European studies (Augustsson, Lindblad, Overvik, & Steineck, 1999; Rohrmann & Becker, 2002).

A number of HAA without the only co-mutagenic β-carbolines have shown to be carcinogenic in long-term exposure studies with rodents and monkeys (Adamson et al., 1990). The International Agency for Research on Cancer classified several HAA as possible (2A) or probable carcinogens (2B) and subsequently recommended a reduced dietary intake of these compounds (IARC, 1993).

These compounds are in particular present in the crust of pan-fried, broiled and grilled meat and fish. The major known HAA, which are formed in meat products during pan-frying under household conditions are the more polar HAA, MeIQx, 4,8-DiMeIQx, and PhIP (Fig. 1). These substances are responsible for most of the observed mutagenic activity (Ames test) in these foods. The co-mutagenic β-carbolines, harmane and norharmane are usually formed under household conditions (Fig. 1). HAA can be divided in two classes, the aminoimidazo-azaarenes and carbolines, especially aminocarbolines. The precursors of aminoimidazo-azaarenes are creatine or creatinine and Maillard products from free amino acids and sugars. They are formed at temperatures below 250 °C. Aminocarbolines are generated as pyrolysis products from amino acids at higher temperatures (>250 °C) with the exception of β-carbolines norharmane and harmane, which are formed at typical frying temperatures. Key factors that influence the formation of HAA are the heating temperature and time (Arvidsson, Van Boekel, Skog, & Jagerstad, 1997; Persson, Oroszvari, Tornberg, Sjoeholm, & Skog, 2008). Moreover, the formation of HAA is influenced by the type of meat, the aging of the meat and the preparation conditions (Melo et al., 2008a; Polak, Andrensek, Zlender, & Gasperlin, 2009; Sinha et al., 1998). A number of publications have demonstrated that frying and broiling are the major processing steps that lead to higher concentrations of HAA (Salmon, Knize, Felton, Zhao, & Seow, 2006).

Several studies have shown that the concentrations of HAA can be reduced by addition of antioxidants such as vitamin E, or extracts of rosemary, garlic, sage, or thyme (Balogh, Gray, Gomaa, & Booren, 2000; Murkovic, Steinberger, & Pfannhauser, 1998; Persson, Graziani, Ferracane, Fogliano, & Skog, 2003; Shin, Strasburg, & Gray, 2002; Tsen, Ameri, & Smith, 2006). For example, the

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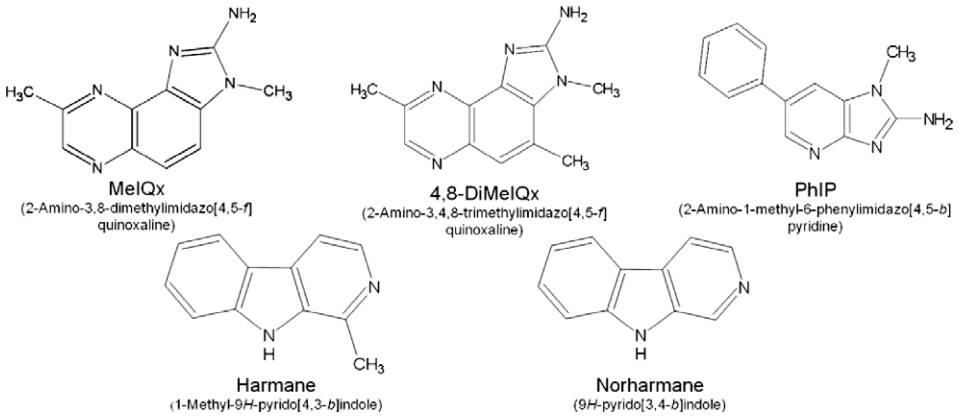

Fig. 1. Formula of commonly found HAA in fried meat products prepared under household conditions.

scavenging effect of antioxidants on pyrazine cation radicals that participate in the formation of HAA has been demonstrated by a decrease in electron spin resonance signals in the present of antioxidants (Kikugawa, 1999). The application of red wine, beer, plant extracts, or combined spice mixtures containing natural antioxidants inhibited formation of HAA when used as marinades prior to frying (Busquets, Puignou, Galceran, & Skog, 2006; Gibis, 2007; Melo, Viegas, Petisca, Pinho, & Ferreira, 2008b; Nerurkar, Le Marchand, & Cooney, 1999; Smith, Ameri, & Gadgil, 2008). This brief pretreatment has the advantage that the products are not overly spiced and do not develope negative sensory characteristics as only the surface is treated.

The objective of this study was to examine the feasibility of inhibiting HAA formation in fried beef patties using marinades containing hibiscus extract. For the determination of the antioxidant capacity, the water phase of marinades with hibiscus extract was analyzed by the TEAC-Assay and the amounts of phenolic compounds determined with the Folin–Ciocalteau-reagent. The sensory quality of the patties after frying was determined using a ranking test, by a trained sensory panel.

2. Materials and methods

2.1. Materials

The HAA-standards 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-5H-pyrido[4,3-*b*] indole), Trp-P-2 (3-amino-1-methyl-5H-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-9H-pyrido[2,3-*b*] indole), MeAαC (2-amino-3-methyl-9H-pyrido[2,3-*b*] indole), harmane (1-methyl-9H-pyrido[3,4-*b*] indole), and norharmane (9H-pyrido[3,4-*b*] indole) with the following end concentrations in the standard mixture 19.7, 22.8, 21.3, 13.6, 12.9, 12.5, 6.5, 5.9, 7.4, 9.4, 5.3 and 5.0 ng in 100 μL methanol, were obtained from Toronto Research Chemicals (Ontario, Canada). All stock solutions of each substance were corrected by means of the extinction coefficient (Hatch, Felton, Stuermer, & Bjeldanes, 1984). Caffeine (internal standard 2.5 μg/mL in ultrapure water and methanol, 1 + 1, v/v), harmane and norharmane (standard mixture: 4.9 and 4.1 ng in 100 μL methanol) were purchased from Sigma–Aldrich (Taufkirchen, Germany). Diatomaceous earth isolute was obtained from Separtis, Germany, and propylsulfonic acid (PRS) (100 mg) and C 18 Bond Elut® cartridges (500 and 100 mg) were purchased from Varian Inc., USA. All other chemicals were analytical or gradient grade for HLPC (Merck, Darmstadt, Germany).

ABTS [2,20-azino-bis(3-ethylbenzothiazoline-6-sulfonate)], Trolox [6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid] and ABAP [2,20-azobis(2-amidinopropane) dihydrochloride] were used for the determination of the antioxidant capacity (TEAC-Assay) and the Folin–Ciocalteu-reagent, respectively. These chemicals were obtained from Sigma (St. Louis, MO, USA).

For the analysis of free amino acids Ala (alanine), Arg (arginine), Asp (aspartic acid), Cys (cysteine), Glu (glutamic acid), Gly (glycine), Ile (isoleucine), Leu (leucine), Lys (lysine), Met (methionine), norleucine, Phe (phenylalanine), Pro (proline), Ser (serine), Thr (threonine), Trp (tryptophan), Tyr (tyrosine), and Val (valine), acetyl chloride, trifluoric acetic anhydride and 2,6-di-tert-butyl-p-cresol were obtained from Sigma (St. Louis, MO, USA), while cation exchanger Dowex 50 WX8 (50–100 mesh) was from Serva (Heidelberg, Germany). 1-Butanol, 2-propanol, diethyl ether, methanol, 2,4,6-trinitrophenol, petroleum ether were supplied by Merck (Darmstadt, Germany).

2.2. Sample materials and marinade formulation

Deep-frozen beef patties were obtained from Salomon Hitburger (Großostheim, Germany) (70 g, 8 mm thick × 113 mm × 105 mm) with the same charge number.

The hibiscus extract (*Hibiscus sabdariffa*) was purchased from Plantextrakt (Vestenbergsgreuth, Germany). Hibiscus extract was used in marinades at concentrations of 0.2, 0.4, 0.6, and 0.8 g per 100 g marinade. For manufacture of the marinades, the water soluble extracts were first dissolved in pure water. Marinade emulsions were then produced by homogenizing sun flower oil, emulsifier (citric acid esters of mono- and diglycerides of fatty acids, E472c, Cognis, Illertissen, Germany) and the respective diluted extract (67.5 g/0.5 g/32 g) using a high-shear disperser (Ultra-Turrax, Janke and Kunkel, Staufen, Germany). In addition to the control marinade that did not contain any extract, a further control was only broiled using sun flower oil.

2.3. Determination of optimal heating time

Two grill plates of a double contact grill (Nevada, Neumärker, Hemer, Germany) were heated to 230 °C. The deep-frozen patties were coated with refined sunflower oil and covered on both sides by tin foil. For the determination of the optimal heating time, the