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Impact of different pan-frying conditions on the formation of heterocyclic aromatic amines and sensory quality in fried bacon

Monika Gibis *, Miriam Kruwinnus, Jochen Weiss

Department of Food Physics and Meat Science, Institute of Food Science and Biotechnology, University of Hohenheim, Garbenstrasse 21/25, 70599 Stuttgart, Germany

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ABSTRACT

Heterocyclic aromatic amines (HAAs) are formed in the crust of cooked meat products. Most HAAs are carcinogenic in long-term animal studies. Besides precursors in raw materials, important factors are temperature and heating time. Bacon slices were investigated for concentrations of HAAs after pan-frying under different monitored heating conditions. Two HAAs, MeIQx (2-amino-3,8-dimethylimidazo [4,5-*f*]quinoxaline) (1.5–5.6 ng/g) and PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine) (0.1–2.6 ng/g), were found in pan-fried bacon slices. The bacon clearly contained higher concentrations of HAAs both with longer frying times and at temperatures of 200–220 °C rather than 150–170 °C, respectively. A similar continuous increase of the concentrations was observed for norharman (5.0–19.9 ng/g) and harman (0.3–1.7 ng/g). The sensory evaluation, using a hedonic test design for colour and flavour, of the pan-fried bacon slices resulted in a preferred frying time of 5 min at 150–170 °C. However, some testers clearly preferred crispy and darker bacon slices containing higher HAA concentrations.

1. Introduction

Several epidemiological studies have shown that a relationship between diet and the risk of the incidence of cancer exists. Red and processed meat particularly focused on the risks of contracting colorectal cancer (WCRF/AICR, 2007). The second expert report by the World Cancer Research Fund (WCRF) and the American Institute for Cancer Research (AICR), in 2007, informed that there is a substantial amount of evidence that red meat and processed meat are convincing causes of colorectal cancer. Nevertheless, this report also noted that there was insufficient evidence to reach any consensus for nitrates, nitrites, heterocyclic aromatic amines (HAAs), nitrosamines, or polycyclic hydrocarbons as risk factors for colorectal cancer. However, the International Agency of Research on Cancer (IARC) has given the recommendation to reduce daily intake of HAAs (IARC, 1993). HAAs are process contaminates which are generated in the Maillard reaction in the crust of meat products as a result of heat treatment. The amounts and ratios of precursors in the raw material, such as creatine, amino acids and reducing sugars, have an important influence on the formation of HAAs (Ahn & Gruen, 2005; Alaejos & Afonso, 2011; Jaegerstad, Skog, Arvidsson, & Solyakov, 1998; Skog, Johansson, & Jagerstad, 1998). The chemical interactions with antioxidants resulted in a reduction of HAA concentration in food (Damasius, Venskutonis, Ferracane, & Fogliano, 2011; Gibis & Weiss, 2012; Johansson & Jaegerstad, 1996; Murkovic, Steinberger, & Pfannhauser, 1998; Persson, Graziani, Ferracane, Fogliano, & Skog, 2003; Puangsombat, Jirapakkul, & Smith, 2011; Vitaglione & Fogliano, 2004) and model systems (Johansson & Jaegerstad,

* Corresponding author. Tel.: +49 711 459 22293; fax: +49 711 459 24446.
E-mail address: gibis@uni-hohenheim.de (M. Gibis).

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1996), and could inhibit the radical reactions in HAA formation which was showed in an electron paramagnetic resonance experiment (Kikugawa et al., 1999). In particular, the preparation method plays a key role in the formation of HAAs; frying and grilling in particular enhance the formation (Keating & Bogen, 2001; Knize, Salmon, Pais, & Felton, 1999). The most influential factors, of each preparation method, are the heating temperature and time (Ahn & Gruen, 2005; Arvidsson, Van Boekel, Skog, Solyakov, & Jagerstad, 1999; Skog et al., 1998). The formation of HAAs is expected to follow a first order reaction equation with the rate constant given by the Arrhenius equation (Arvidsson, Van Boekel, Skog, & Jagerstad, 1997; Arvidsson et al., 1999). Although meat and patties have been widely investigated with regard to their HAA content, only a small database exists about the common concentrations of HAAs for processed meat, such as bacon, sausages or ready-to-eat meat products (Puangsombat, Gadgil, Houser, Hunt, & Smith, 2011). In a recent study, salami and ham slices as pizza toppings were investigated, and sudden increases of HAA concentrations were found after exceeding specific temperatures and times (Gibis & Weiss, 2013). A recent review gives a detailed overview of this occurrence in meat products (Alaejos & Afonso, 2011). HAA levels for pan-frying of fried bacon were studied by some authors (Back, Lee, Shin, & Lee, 2009; Gross et al., 1993; Guy, Gremaud, Richoz, & Turesky, 2000; Johansson & Jaegerstad, 1994; Ni, McNaughton, LeMaster, Sinha, & Turesky, 2008; Puangsombat et al., 2011), but in most cases the bacon slices were bought from a grocery, so the raw material and thereby precursors may differ in the frying experiments.

The objective of this study was the analysis of bacon slices concerning the concentration of HAAs after different monitored heating treatments. For this objective, the bacon was manufactured from belly without rind and gristle. As a working hypothesis, the concentration of HAAs may be affected by the pan-frying time and temperature. The bacon slices were manufactured according to the manufacturing process defined from one animal to exclude other factors of influence. The sensory preference of the cooked meat product is also a very important factor for the consumer intake of HAAs. In order to study this consumer preference, the sensory quality of the bacon slices according to colour and flavour, as well as liking, after pan-frying was determined using a hedonic test with a trained sensory panel.

2. Materials and methods

2.1. Chemicals

Norharman, harman and caffeine were purchased from Sigma-Aldrich (Taufkirchen, Germany). 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 were obtained from Toronto Research Chemicals (North York, Canada). Ultrapure water was produced on-site using an ultrapure water system (Herco, Freiberg, Germany). Aqueous ammonia (25%), acetonitrile (gradient grade), methanol (gradient grade), toluene, and ethyl acetate (gradient grade), were obtained from Carl Roth (Karlsruhe, Germany), and hydrochloric acid, ammonium acetate, sodium hydroxide, orthophosphoric acid, and triethylamine from VWR International (Darmstadt, Germany). All chemicals were analytical grade.

2.2. Apparatus and materials

An HPLC system (Gynkotek, Germering, Germany), consisting of a M480 pump, Gina 50 autosampler, DG 1310 S degasser, RF 1002 fluorescence detector, UVD 320 diode array detector, and column oven (Thermo Technic Productions, Langenzersdorf, Österreich), was used. Gynkosoft chromatography data system Version 5.50 was applied for data acquisition. The following items were used for HAA analysis: Ultra Turrax T-25 (IKA Labortechnik, Staufen, Germany), a centrifuge Biofuge 28 RS (Heraeus Sepatech, Osterode, Germany), an evaporator Barkley Model BB 74300 (Leopoldshöhe, Germany), a Model HP 8453 spectral photometer (Agilent Technologies, Waldbronn, Germany), solid phase extraction cartridges Bond Elut® PRS (propylsulfonic acid cation exchanger), Bond Elut C18, 100 mg, 500 mg, as well as (Varian, Palo Alto, CA) diatomaceous earth bulk sorbent Isolute HM-N, extraction blank cartridges Isolute® from Separtis, (Grenzach-Wyhlen, Germany), a filter type 0967, 11 mm ID (Schleicher & Schuell, Dassel, Germany), and an analytical column TSK-gel ODS-80 250 × 4.6 mm, 5 µm (Tosoh Bioscience, Stuttgart, Germany), connected to a guard column Supelguard™ LC-18-DB (Supelco, Bad Homburg, Germany). The following equipment was used for the other determinations: extraction unit Büchi 810 (Büchi, Flawil, Switzerland) for fat, hydrolysing unit Büchi-425, and digestion unit Büchi-323 (Büchi, Flawil, Switzerland) for protein analysis, and Aqua Lab CX-2 (Decagon Devices Inc., Pullman, WA, USA) for measurements of water activity (a_w).

2.3. Bacon preparation

Belly without rind and gristle (approximately 2.5 kg initial weight) was used, and 40 g/kg curing salt (0.9% sodium nitrite, 99.1% sodium chloride), 5 g/kg bacon seasoning, 3 g/kg Schinkin® Cum Spezial (Reinert Gruppe Ingredients GmbH, Erftstadt, Germany) as curing aids, containing dextrose, monosodium glutamate, sodium ascorbate, sodium isoascorbate, maltodextrin, flavouring and 0.25 g/kg starter cultures (Bitec SM 96, Frutarom, Ditzingen, Germany). The preparation of the bacon was a dry-curing process for 4 days. After removal of the excess curing salt and a ripping time of 5 days (+2 °C, 75–80% rel. humidity), the bacon was dried for 4 h and smoked for 10 min in a universal smoking and heating chamber (Unigar 1800 BE Compact with software Ness Digitronic 4, Ness & Co. GmbH, Remshalden, Germany) using a smoking programme with 5 min cold smoke, and 5 min smoke settling. The pieces were dried again at 28 °C for 5 min and the process was repeated for up to 85.5% of the initial weight. Each piece of bacon was sliced into slices 1.5 mm thick. The slices were stored in modified atmosphere packaging (20% N_2 and 80% CO_2) at 5 °C until they were pan-fried.

2.4. Heating conditions

The bacon slices were pan-fried for 3, 4, 5, and 6 min at 150–170 °C, as well as 2 and 3 min at 200–220 °C in a Teflon pan which was rubbed with sunflower oil before using. The pan was cleaned after each frying procedure and was rubbed again with sunflower oil. The temperature was monitored by using a data logger (Therm 3280-8M, Ahlborn, Holzkirchen, Germany).

The bacon slices were continuously turned over after 1 min and 30 s, respectively, for last flip. The total pan-frying time for each side of each slice was equal. The heated bacon slices were then cooled and stored at 4 °C until use in subsequent analysis.

2.5. Determination of HAAs

The method includes the determination of polar and apolar HAAs. The method of HPLC analysis (Gibis, 2007) used, with some modifications, was based on the method described by Gross and Grueter (1992). The bacon slices ($n = 8$) were cut with a blender and homogenised (approximately 16.5 g) with 90 ml sodium hydroxide solution (1 mol/l) using an Ultra-Turrax. Diatomaceous earth was added to each of the four equivalent amounts of homogenates and mixed. Two of the homogenates had been previously