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Inhibitory effect of hawthorn extract on heterocyclic aromatic amine formation in beef and chicken breast meat

Mercan Merve Tengilimoglu-Metin[a,*], Aytul Hamzalioglu[b], Vural Gokmen[b], Mevlude Kizil[a]

[a] Department of Nutrition and Dietetics, Faculty of Health Sciences, Hacettepe University, 06100, Sihhiye, Ankara, Turkey
[b] Food Quality and Safety (FoQuS) Research Group, Department of Food Engineering, Faculty of Engineering, Hacettepe University, 06800, Beytepe, Ankara, Turkey

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IQ (PubChem CID: 53462)
IQx (PubChem CID: 105041)
MeIQ (PubChem CID: 62274)
MeIQx (PubChem CID: 62275)
7,8-DiMeIQx (PubChem CID: 104855)
PhIP (PubChem CID: 1530)
Harman (PubChem CID: 486-84-0)
Norharman (PubChem CID: 244-63-3)
AαC (PubChem CID: 62805)
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ABSTRACT

This study focused on the inhibitory effect of different levels of hawthorn extract (0, 0.5, and 1%) on the formation of heterocyclic aromatic amines (HAAs) in beef and chicken breast cooked by either pan-cooking or oven-cooking. All meat samples were cooked at three different temperatures (150, 200, and 250 °C) and the levels of twelve HAAs were assessed (IQ, IQx, MeIQ, MeIQx, 4,8-DiMeIQx, 7,8-DiMeIQx, PhIP, harman, norharman, AαC, MeAαC, and Trp-P-2). Varying levels of IQ (up to 4.47 ng/g), IQx (up to 0.69 ng/g), MeIQ (up to 0.82 ng/g), MeIQx (up to 1.01 ng/g), 4,8-DiMeIQx (up to 0.10 ng/g), 7,8-DiMeIQx (up to 0.23 ng/g), PhIP (up to 0.75 ng/g), harman (up to 2.15 ng/g), norharman (up to 1.08 ng/g), AαC (up to 1.86 ng/g), MeAαC (up to 0.48 ng/g), and Trp-P-2 (up to 12.88 ng/g), were detected. Samples cooked at 150 °C had very low amounts of HAAs, and the levels of HAAs increased gradually when the cooking temperature rose from 150 to 250 °C. The total HAA content in chicken breast and beef ranged between not detectable to 17.60 ng/g, and not detectable to 11.38 ng/g, respectively. The inhibitory effects of hawthorn extract at 0.5% and 1% on total HAAs levels were found to be 12–100% and 19–97% in chicken breast, respectively, and 42–100% and 20–35% in beef, respectively. This study demonstrated that hawthorn extracts at 0.5% and 1% could mitigate HAA formation, especially at high cooking temperatures.

1. Introduction

Heterocyclic aromatic amines (HAAs) are compounds that are formed naturally during the cooking of protein rich foods such as beef, pork, fish, and poultry (Oz, Kaban, & Kaya, 2010). HAAs were first discovered by Sugimura (1997) and to date more than twenty-five different HAAs have been isolated and identified in cooked protein rich food (Sanz Alaejos, Ayala, González, & Afonso, 2008). HAAs are recognized as being mutagenic and/or carcinogenic compounds that are present at parts per billion levels in cooked meat. The International Agency for Research on Cancer (IARC) has classified MeIQx, MeIQ, and PhIP AαC, MeAαC, Trp-P-1, Trp-P-2, Glu-P-1 and Glu P-2 as "reasonably anticipated to be a human carcinogen" in class 2B, while IQ has been placed on the list of "probable human carcinogen" in class 2A (IARC, 1993). HAAs are formed as a result of a complex Maillard reaction between creatine/creatinine, free amino acids, and reducing sugars (glucose and fructose, directly or via hydrolysis of sucrose), which are naturally present in mammalian muscular tissue, at cooking temperatures over 150 °C (Haskaraca, Demirok, Kolsarıcı, Oz, & Ozsarac, 2014). The factors that influence the formation of HAAs include physical factors such as meat type, amount of meat, cooking method, cooking temperature, cooking duration, cooking equipment, meat acidity (pH), and water activity, duration of storage of fresh meat, as well as the levels of precursors in the meat (carbohydrates, free amino acids, creatine/creatinine, and amino acids) (Knize & Felton, 2005; Oz, Kizil, Zaman, & Turhan, 2016; Szterk & Waszkiewicz-Robak, 2014; Zhang, Yu, Mei, & Wang, 2013). HAA concentrations are also affected by lipid content, lipid oxidation, and the presence of

Abbreviations: HAA, heterocyclic aromatic amine; nd, non-detected; 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; AαC, 2-amino-9H-pyrido[2,3-*b*]indole; MeAαC, 2-amino-3-methyl-9H-pyrido[2,3-*b*]indole; Norharman, 9H-pyrido[3,4-*b*]indole; Harman, 1-methyl-9H-pyrido[4,3-*b*]indole; Trp-P-2, 3-amino-1-methyl-5H-pyrido[4,3-*b*]indole; 4,7,8-TriMeIQx, 2-amino-3,4,7,8-tetramethylimidazo[4,5-*f*]quinoxaline

* Corresponding author.
E-mail addresses: tengilimoglu@hacettepe.edu.tr (M.M. Tengilimoglu-Metin), aytulhamzalioglu@hacettepe.edu.tr (A. Hamzalioglu), vgokmen@hacettepe.edu.tr (V. Gokmen), mkizil@hacettepe.edu.tr (M. Kizil).

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antioxidants (Oz & Kaya, 2011b). It is recommended that human exposure to these compounds should be minimized (Dong, Lee, & Shin, 2011; Gibis & Weiss, 2010) and identifying effective inhibitors of HAAs is an important goal. In this regard, the addition of antioxidants to meat prior to cooking has shown to be effective in reducing HAA levels. It has been suggested that free radical reactions play an important role in HAA-forming pathways, through the Maillard reaction, and that antioxidants may act as free radical scavengers in the early stages of the Maillard reaction thereby decreasing HAA formation (Zeng et al., 2014).

Hawthorn, a common name for all plant species in the genus *Crataegus* of the *Rosaceae* family, grows in Northern Europe, North America, and Asia (Chang, Zuo, Chow, & Ho, 2006). There are 165–200 hawthorn species worldwide and about seventeen hawthorn species are found in Turkey (Gundogdu et al., 2014). The most commercially used species is *C. pinnatifida* (Liu, Yang, & Kallio, 2010). Hawthorn is commonly used as a medicinal plant in many countries due to its cardiotropic, anti-arrhythmic and vasodilatory effects. Hawthorn extracts are industrially produced from the fruit, flowers, and leaves of the hawthorn plant and the major functional compounds are phenolics, including flavonoids and pro-anthocyanins (Keating et al., 2014; Liu et al., 2010), but they also contain chlorogenic acid, epicatechin, hyperoside, quercetin, rutin, vitexin, rhamnoside, and procyanidins that also have antioxidant properties (Shortle et al., 2014).

Several studies have so far showed the inhibitory effects of synthetic or natural antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) (Ahn & Grun, 2005) and propyl gallate (PG) (Johansson & Jägerstad, 1996), fruit or plant extracts (Gibis & Weiss, 2012; Tsen, Ameri, & Smith, 2006), onions (Gibis, 2007), tomatoes (Vitaglione, Monti, Ambrosino, Skog, & Fogliano, 2002), cherry tissue (Britt, Gomaa, Gray, & Booren, 1998), green tea (Quelhas et al., 2010), vitamin E (Ruan et al., 2014), wine (Busquets, Puignou, Galceran, & Skog, 2006), beer (Melo, Viegas, Petisca, Pinho, & Ferreira, 2008), and various spices (Zeng et al., 2014) on HAA formation in meat and meat products. However, to the best of our knowledge, the effect of the addition of hawthorn extract on the formation of HAAs has not been investigated. The aim of the present study was to investigate the effect of hawthorn extract on HAA levels in pan-fried and oven-cooked, beef and chicken breast meat, when cooked at different temperatures.

2. Material and methods

2.1. Materials

2.1.1. Raw materials

The chicken breast meat and beef (*M. longissimus dorsi*) muscle were purchased from a local market in Ankara, Turkey. They were transported to the laboratory on ice. The commercial hawthorn extract (*C. pinnatifida*) was obtained from Balen, Ankara, Turkey.

2.1.2. Chemicals

Propyl gallate (PG), ethylenediaminetetraacetic acid disodium (EDTA), tetraethoxypropane (TEP), trichloroacetic acid (TCA), thiobarbituric acid (TBA), diacetyl, diethyl ether, picric acid, 1-naphthol, creatine standard, creatinine standard, potassium hexacyanoferrate, zinc sulfate, D-(+)-glucose (99.5%) and D-(+)-fructose (99%), ABTS (2,20-azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid)), Folin-Ciocalteu reagent, Potassium peroxydisulfate, 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), gallic acid were obtained from Merck (Darmstadt, Germany). Chemicals for HAA analysis, including ethyl acetate, methanol, acetone, sodium hydroxide, hydrochloric acid, glacial acetic acid, acetonitrile, and ammonium hydroxide solution (25%) were from Merck. For solid phase extraction, Extrelut NT packaging material (Merck, Darmstadt, Germany), Bond Elut reservoir (Varian, Harbor City, California, USA), Oasis MCX cartridge (Waters, Milford, Massachusetts, USA), SPE manifold (Supelco Visiprep, St. Louis, Missouri, USA), Oasis HLB cartridge (Waters, Milford, Massachusetts, USA) were used. Heterocyclic aromatic amine standards were purchased from Toronto Research Chemicals (Downsview, Ontario, Canada): IQ (CAS no:76180-96-6, 2-amino-3-methylimidazo [4,5-*f*]quinoline), IQx (CAS no:108354-47-8, 2-amino-3-methylimidazo [4,5-*f*]quinoxaline), MeIQ (CAS no:77094-11-2, 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline), MeIQx (CAS no:77500-04-0, 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline), 4,8-DiMeIQx (CAS no:95896-78-9, 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline), 7,8-DiMeIQx (CAS no:92180-79-5, 2-amino-3,7,8-trimethylimidazo[4,5-*f*]quinoxaline), PhIP (CAS no:105650-23-5, 2-amino-1-methyl-6-phenylimidazo [4,5-*f*]pyridine), harman, (CAS no:486-84-0, 1-methyl-9H-pyrido[3,4-*b*] indole), norharman (CAS no:244-63-3, 9H-pyrido[3,4-*b*]indole), AαC (CAS no:26148-68-5, 2-amino-9H-pyrido[2,3-*b*]indole), MeAαC (CAS no: 68006-83-7, 2-amino-3-methyl-9H-pyrido[2,3-*b*]indole), Trp-P-2 (CAS no:72254-58-1, 3-amino-1-methyl-5H-pyrido[4,3-*b*] indole) and 4,7,8-TriMeIQx (CAS no: 132898-07-8, 2-amino-3,4,7,8-tetramethylimidazo[4,5-*f*]quinoxaline). Chemicals and solvents were of high-performance liquid chromatography (HPLC) or analytical grade. All solutions, except for HPLC-grade solutions, were passed through a 0.45 μm filter (Millipore, Billerica, Massachusetts, USA) before use.

2.2. Methods

2.2.1. Sample preparation

All meat samples were sliced to a thickness of 1 cm and divided into 36 groups according to meat type (beef, chicken breast meat), cooking methods (pan-frying, oven-roasting), cooking temperatures (150, 200, and 250 °C) and hawthorn extract concentrations (0, 0.5, and 1.0%). Twelve of these groups were chosen as control groups. All experiment were performed in two replicates and two slices (approximately, 100 g/slice) of meat sample were used for per treatment. The water soluble hawthorn extracts were prepared in distilled water (1/10) (w/v) at concentrations of 0 (control), 0.5 and 1% (w/w) and the mixture was rubbed equally on each surfaces of the meats. After adding the extract, the samples were stored at 4 °C overnight.

2.2.2. Cooking process

Both pan-frying and oven-roasting cooking methods were used. The pan-frying process was carried out with a Teflon-coated pan. The cooking time was determined based on preliminary experiments. All samples were prepared without salt, spices, fat, or oil. For pan-frying, samples were cooked for 5 min per side. For oven-roasting, samples were placed in an oven and cooked for 20 min. Three different temperatures (150, 200, and 250 °C) were used for both cooking methods and the surface temperature was measured using a thermometer (Testo 905-T2, Lenzkirch, Germany) to adjust the cooking temperatures. After cooking, the samples were cooled at room temperature, weighed, and homogenized using a kitchen blender (Tefal, Sarcelles, France) to produce a uniform sample and were stored at − 20 °C until analysis.

2.2.3. Proximate composition, pH analysis, and cooking loss

The proximate composition of meat samples, including protein, lipid, ash, moisture content was performed according to AOAC methods (Horwitz, 2000). The lipid content was determined by the Soxhlet method and the protein content was analyzed by the Kjeldahl method. The pH of samples was measured using a digital pH meter (Hanna, Vohringen, Germany) calibrated with standard buffers of pH 4.0 and 7.0 at room temperature. The sample losses of weight during cooking were calculated from the differences in raw and cooked weight. All parameters were determined in duplicate.

2.2.4. Creatine/creatinine analysis

The contents of creatine and creatinine in the meat samples were