

THE INHIBITION OF HETEROCYCLIC AROMATIC AMINE (HAAs) FORMATION AND MODIFICATION OF THE VOLATILE FLAVOR PROFILE IN ROAST BEEF: A REVIEW

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Abstract

Heterocyclic aromatic amines (HAAs) are potential carcinogens that are formed during thermal processing, particularly when meat is exposed to elevated temperatures such as grilling or roasting. The importance of ingredients and marinades, rich in antioxidants properties (like tea, bay leaf, star anise, red chili, oregano), for inhibit the formation of these harmful compounds. Reducing lipid oxidation and Maillard reaction compounds formation, while improving the nutritional quality, safety and sensory preference of grilled meats. The aim of this study was to determine the inhibitory effects of natural and synthetic antioxidants, seeds, spices, fruit, and plant extracts on HAA formation in roast beef meat.

Key words: antioxidants, beef, carcinogen, flavour, heterocyclic aromatic amines.

INTRODUCTION

According to data on nitrogen balance, the European Food Safety Authority (EFSA) recommends an average daily protein intake for adults - both men and women - is 0.66 g per kilogram of body weight; for newborns, it can reach up to 1.12 g per kg of body weight (EFSA, 2021). Reference Values suggest that this is the lowest level of consumption needed to satisfy the advised intake for half of the healthy individuals. Heat processing is the most common method for consuming beef and is considered the last component influencing the ultimate quality of meat (Tkacz et al., 2021; Gil et al., 2022). Generally, meat products provide particular nutrients in our daily diet (Teng et al., 2023). They are an abundant source of folic acid, vitamins B6 and B12, and proteins in the human body (Kulczyński et al., 2019). Trace elements include iron, magnesium, selenium, copper, zinc, essential amino acids, and lipids (Bohrer, 2017). Primarily mutagenic and cancer-causing heterocyclic aromatic amines (HAAs) are formed in high-temperature processed foods that are high in protein, including meat, fish, and cooked foods (Chen & Xi, 2022; Yang et al., 2021). Maillard reactions and the pyrolysis

of proteins and amino acids are responsible for the formation of HAAs (Jing et al., 2021; Teng et al., 2023). Food safety research has focused on preventing the formation of hazardous substances during high-temperature food preparation (Quan et al., 2022). Epidemiological studies have shown that consumption of foods containing HAAs significantly increases the risk of cancer (Cheng et al., 2023; Meurillon et al., 2020).

MATERIALS AND METHODS

For this study, we conducted a systematic search of scientific publications in the Elsevier, ScienceDirect, Scopus, Springer Nature, Web of Science, MDPI and Wiley databases. This review can provide theoretical reference and technical support for developing strategies to effectively inhibit heterocyclic aromatic amines (HAAs) generation and modification of the volatile flavor profile in roast beef meat to the understanding for consumers and relative food processors.

RESULTS AND DISCUSSIONS

HAAs FORMATION

Meat products are rich in carbohydrates, proteins, amino acids, creatine, creatinine, and

other components, which form heterocyclic aromatic amines (Zhang et al., 2025). Consumers appreciate the particular taste of roasted beef acquired during thermal processing, which is considered to be the main process for flavor creation. The Maillard reaction results from the interaction of amino compounds and reducing sugars, which provides food-rich flavors and sensory qualities (Chu et al., 2025). When meat is cooked at high temperatures, the Maillard reaction results in hazardous substances called HAAs, which are affected by various factors (Nadeem et al., 2021). Broasted beef with ascorbic acid, protein oxidation, caramelization, lipid oxidation, and the Maillard reactions are all connected (Oz et al., 2023). Strecker degradation produces pyrazine flavor compounds in the final stage of MR, where heterocyclic chemicals such as heterocyclic aromatic amines are formed (Wang et al., 2023).

CLASSIFICATION OF HAAs

HAAs were initially found on the surface of grilled fish and roast beef (Oz et al., 2023). More than 30 HAAs have been evaluated in all types of heated foods (Gibis, 2016). Heterocyclic aromatic amines (HAAs) of thermal origin are synthesized through the interaction between proteins and free amino acids at temperatures ranging from 100°C to 300°C. In contrast, pyrolytic HAAs are generated at temperatures surpassing 300 °C.. The primary classification of these two types is largely based on the reactions that lead to their formation (Nadeem et al., 2021). Further research focusing on the structure, temperature, and manufacturing methods has classified polar HAAs (IQ type) and non-polar HAAs (non-IQ type) into two primary categories. Polar HAAs are formed from amino acids, creatine, creatinine, carbohydrates at temperatures between 150-250°C, reducing sugars, and other precursors (Puangsombat et al., 2012; Yu et al., 2016; Yao et al., 2023). Nonpolar HAAs are formed from pyridoindole or dipyridoimidazole via the pyrolysis of amino acids at higher temperatures (Karpavičiūtė et al., 2017). In 2025, Mirza Alizadeh et al. divided heterocyclic aromatic amines into two distinct groups: polar (amino-imidazoazarenes) and non-polar (amino-carbolines). The pyridines, quinolines, and quinoxalines, are all polar

HAAs. Non-polar HAAs, in Table 1, represent the α-amino-carboline, β-amino-carboline, γ-amino-carboline, and ε-amino-carboline groups.

Table 1. Several of the polar and non-polar heterocyclic aromatic amines, data processed after Mirza Alizadeh et al. (2025)

Group	HAA	Chemical name
Quinolines	IQ	2-Amino-3-methylimidazo[4,5-f]quinolone
	MeIQ	2-Amino-3,4-dimethylimidazo[4,5-f]quinoline
Quinoxalines	IQx	2-Amino-3-methylimidazo[4,5-f]quinoxalines
	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
	4,7,8-TriMeIQx	2-Amino-3,4,7,8-tetramethylimidazo [4,5-f]quinoxaline
Pyridines	PhIP	2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine
	DMIP	Dimethylimidazopyridine
	TMIP	Trimethylimidazopyridine
α-amino-carbolines	AαC	2-Amino-9H-dipyrdo[2,3-b]indole
	MeAαC	2-Amino-3-methyl-9H-dipyrdo[2,3-b]indole
β-amino-carbolines	NorHarman	9H-Pyrdo[3,4-b]indole
	Harman	1-Methyl-9H-pyrdo[3,4-b]indole
γ-amino-carbolines	Trp-P-1	3-Amino-1,4-dimethyl-5H-pyrdo[4,3-b]indole
	Trp-P-2	3-Amino-1-methyl-5H-pyrdo[4,3-b]indole
δ-amino-carbolines	Glu-P-1	2-Amino-6-methyl-dipyrdo[1,2-a:3',2'-d]imidazole
	Glu-P-2	2-Amino-dipyrdo[1,2-a:3',2'-d]imidazole

EFFECTS ON HUMAN HEALTH

Over the past few decades, the formation of heterocyclic aromatic amines at elevated temperatures has been documented, raising concerns about various health hazards, including their carcinogenic potential (Teng et al., 2023). Since 1993, the International Agency for Research on Cancer (IARC) has been involved in the classification of numerous HAAs. Containing "MeIQ, MeIQx, PhIP, AαC, MeAαC, Trp-P-1, Trp-P-2 and Glu-P-2" as

"Group 2B, possible human carcinogens" and a HAA (IQ) as "Group 2A, probably human carcinogen". Studies have shown that HAA exposure increases the risk of lung, pancreatic, breast, colon, colorectal, and stomach cancer (Barzegar et al., 2019; Bellamri et al., 2021; Oz et al., 2023). PhIP, IQ, MeIQx, and MeIQ have been identified by the National Toxicology Program of the US Department of Health and Human Services as expected human carcinogens (Chen et al., 2019). Experiments with Ames *Salmonella typhimurium* have shown that most HAAs have mutagenic potentials (Khan et al., 2024). HAAs have been reported to be over 100 times more dangerous than aflatoxin B1, and more than 2000 times more harmful than benzo[*a*]pyrene (Huang et al., 2025).

METHODS OF INHIBITING HAAs

The development of strategies to mitigate the effects of HAA exposure has led to research on the modification of cooking methods and the use of seeds, spices, plant extracts, and fruits (Geng et al., 2024; Oz et al., 2023). Natural antioxidant compounds may influence HAA formation by reducing lipid oxidation (Chu et al., 2024). The radical Maillard reaction pathways can be modified by antioxidants, thereby reducing the productivity of the HAA formation reaction (Cao et al., 2020). Extracts from seeds (Khan et al., 2024), reishi mushroom (Guzel et al., 2024), inhibition mechanism of tea polyphenols (Wang et al., 2024), white and oolong tea (Caliskan et al., 2023), ginseng (Yao et al., 2024), citrus peel (Xu et al., 2023), pomegranate peel (Chen et al., 2023), mulberry leaf extract (Xu et al., 2024), and bitter melon extract *Momordica Charantia* (Gumus et al., 2025) have demonstrated inhibitory effects on the generation of HAA in different types of meat. Antioxidants have been shown to lower the production of HAAs by blocking different pathways of formation. They scavenge free radicals and trap precursors to lower the levels of HAAs in cooked meat (Bulanda et al., 2025). Previous studies have investigated the inhibitory effects of natural and synthetic antioxidants, seeds, spices, fruit, and plant extracts on HAA formation in cooked beef or

marinades, several of which are summarized in Table 2.

Table 2. Some of the types of inhibitors and HAAs inhibitions % in cooked beef

Types of Inhibitors	HAAs inhibitions %	Reference
Nutmeg (Seed) Antioxidants	MeIQ 100%	Teng et al. (2023)
Turmeric (Root) Curcumin, Desmethoxycurcumin, Bisdsmethoxycurcumin	IQ, IQx, 7.8 DiMeIQx, PhIP, Harman, Norharman, AaC 75%	Mahmood (2019)
Sichuan pepper (seed) Phenolic compounds	PhIP 82% IQx 61% MeIQx 28% 4,8Di MeIQx 79%	Zeng et al. (2018)
Turmeric and lemongrass (marinate) Phenolic compounds	IQ, PhIP, Harman, Norharman, AaC 94.7%	Sepahpour et al. (2018)
Pomegranate (seed) Tocopherols, phenolic acids (hydroxybenzoic acid)	IQ, 65% PhIP, 65% MeIQx, 59% Norharman, 69%	Keskekoglu & Uren (2017)
Hawthorn Phenolic compounds Flavonoids Proanthocyanins	IQx, IQ, MeIQx MeIQ, 4.8 DiMeIQx, 7.8 DiMeIQx, PhIP, Harman, Nor-Harman, MeAaC, TrP2, 20–100%	Tengilimoglu-Metin et al. (2017)
Artichoke (Flower buds) Mono- and di-caffeoylquinic acids, flavonoids	IQx, IQ, MeIQx, AaC MeIQ, 4.8 DiMeIQx, 7.8 DiMeIQx, PhIP, Harman, TrP2, Meac NorHarman 25-98%	Tengilimoglu-Metin & Kizil (2017)
Apple, elderberry, grape seed, pineapple Proanthocyanidins, phloridzin, and chlorogenic acid	PhIP, MeIQx, 4,8-DiMeIQx 70%	Cheng et al., (2007)
Apple peel powder Polyphenol	PhIP, 83%, MeIQx: 68; 4,8DiMeIQx, 56%	Sabally et al. (2016)
Grape (Seed) Procyanidins polyphenols	MeIQx, 70% PhIP, 90%	Ahn & Grün (2006)
Savory (leaves) Phenolic compounds	PhIP 37.31%	Damašius et al. (2011)
Oregano (leaves) Phenolic compounds	PhIP 43.28%	Damašius et al. (2011)
Rosemary (Leaves) Rosmarinic acid, carnosol, carnosic acid	MeIQx, 91.7% PhIP, 85.3%	Quelhas et al. (2010)
Hibiscus (marinated) Phenolic compounds	MeIQx 50%	Gibis & Weiss (2010)
Hydrocolloids κ-carrageenan	PhIP, 90% MeIQx, 64% 4,8DiMeIQx, 48%	Yang et al. (2021)
Blueberry Phenolic contents	IQx, IQ, MeIQx, AaC, MeIQ, 7.8 DiMeIQx, Harman, TrP2, 91-98%	Gumus & Kizil (2022)
<i>Rosa rugosa</i> tea (RTE) Phenolic compounds	MeAaC, AaC PhIP, Harman, NorHarman, TrP1, TrP2 75%, 46%	Jamali et al. (2016)
<i>Portulaca oleracea</i> L. (PO) Flavonoid	Harman, NorHarman, Glu-P-1 62.39/68.03/73.75%	Jing et al. (2021)
<i>Momordica charantia</i> L. extract (MCE) Antioxidant	PhIP, IQ, IQx, MeIQ, MeIQx, 4,8-DiMeIQx, 7,8- DiMeIQx, AaC, 30.08%	Gumus et al. (2025)
Bay leaf, star anise, and red chili (spices) Capsaicin (phenolic)	Harman, 78.08% NorHarman, 64.11%	Zhou et al. (2024)
Bay leaf, star anise, and red chili (spices) Kaempferol (phenolic)	PhIP, 80.87%	Zhou et al. (2024)

INFLUENCE OF THE STORAGE ON HAAs

The general sensory quality of the beef that was stored at 2°C for 5 and 15 days was elevated, while the levels of IQ, MeIQx, MeIQ, Phe-P 1, and AαC were relatively low during the processing (Szterk & Jesionkowska, 2015). Improving preservation and storage conditions is one strategy to reduce the accumulation of HAAs in beef production (Lai et al., 2023).

METHODE TO QUANTIFICATION AND IDENTIFICATION HAA

Effective sample preparation procedures and instrumental methods applied for the detection and separation of HAAs (usually µg kg⁻¹ or ppb) are required to improve their accuracy and detectability before instrumental analysis (Barzegar et al., 2019; Cao et al., 2020). The most often used method for analyzing HAAs is high-performance liquid chromatography coupled with mass spectrometry (HPLC-MS/MS) (Erdoğan & Özdestan-Ocak, 2022; Gumus et al., 2025).

Combining gas chromatography (GC) and enzyme-linked immunosorbent assays (ELISA) with a detector is one way to identify hazardous compounds (Nadeem et al., 2021; Bulanda et al., 2025).

CONCLUSIONS

To reduce the health risks associated with HAA exposure, efforts are being made to change dietary habits and food processing methods. By employing differential strategies, it is possible to lower the concentration of HAAs in foods cooked at high temperatures, thereby creating food products that are both healthier and safer by preventing the formation of HAAs.

People generally steer clear of meats that have been heavily processed, particularly when these meats are cooked using high-temperature techniques like frying, roasting, or grilling. Studies should be conducted to investigate the implementation of this strategy, which not only significantly reduces the content of HAAs in meat products, but also protects public health by reducing the intake of harmful carcinogens by consumers in their daily diet.

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