

Research Article

Influence of Garlic Marinade on Maillard Reaction and Polycyclic Aromatic Hydrocarbon Formation in Grilled Chicken

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ABSTRACT

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Garlic, a common ingredient in Thai cuisine, is a natural source of antioxidants and may influence chemical reactions during cooking, such as the Maillard reaction and the formation of polycyclic aromatic hydrocarbons (PAHs), which are potential carcinogens. This study investigated the effect of garlic-containing marinades on these processes in grilled chicken. Marinades with 8%, 15%, and 26% garlic were prepared, and chicken breasts were marinated for 12 hours. A non-marinated sample served as the control. All samples were grilled on a hotplate at 350 °C for 10 minutes. Antioxidant activity of the marinades and marinated chicken was assessed using DPPH assay (2,2-diphenyl-1-picrylhydrazyl) and ABTS assay (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), and total phenolic content was measured. The Maillard reaction was evaluated through color change and melanoidin absorbance, while 16 PAHs were quantified using Gas Chromatography–Mass Spectrometry. The results showed that higher garlic concentrations enhanced the antioxidant activity of the marinades but did not significantly affect Maillard reaction markers or PAH formation. The limited PAH levels observed are likely due to the grilling method—specifically, the use of a hotplate heat source, its temperature, and the cooking duration—which may not induce substantial PAH production. These findings suggest that garlic enhances antioxidant properties in marinades but may have limited impact on PAH mitigation under the cooking conditions used.

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1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of chemical compounds primarily formed during the incomplete combustion of organic matter. In the context of cooking, they are commonly produced when fat and meat juices drip onto hot surfaces, such as charcoal, generating smoke that carries PAHs which then deposit onto the food (Janoszka, 2020). Numerous studies have confirmed that PAH formation is significantly influenced by cooking temperature, time, and method. For example, charcoal grilling typically produces higher PAH levels than electric or gas grilling due to direct flame and smoke exposure (Yurchenko and Mölder, 2021). Meat type and fat content also play a crucial role; fattier meats

tend to generate more smoke, resulting in higher levels of PAHs (Ni *et al.*, 2021).

PAHs are chemically inert and hydrophobic, but once ingested, they undergo metabolic transformation in the human body into reactive metabolites. These metabolites can bind covalently to DNA, leading to mutations and replication errors that initiate carcinogenesis (Phillips, 1999). Due to their toxicity and health risks, the U.S. Environmental Protection Agency (US EPA) has identified 16 PAHs as priority pollutants. Similarly, the European Union's Scientific Committee on Food has designated four PAHs: benzo[a]anthracene (B[a]A), chrysene (Chr), benzo[b]fluoranthene (B[b]F), and benzo[a]pyrene (B[a]P), as key indicators of carcinogenic PAHs in food (Lee *et al.*, 2016).

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Several strategies have been studied to reduce PAH formation in grilled meats. Farhadian *et al.* (2011) demonstrated that preheating and wrapping beef or chicken before charcoal grilling could completely eliminate benzo[a]pyrene and benzo[b]fluoranthene formation. Recent studies highlight the effectiveness of marinades, particularly those rich in antioxidants such as lemon juice, garlic, or wine in significantly reducing PAH levels by inhibiting fat and protein pyrolysis (Zhang *et al.*, 2022). In addition to reducing PAHs, marination can also lower the formation of heterocyclic aromatic amines (HCAs), another class of carcinogens generated during high-temperature cooking (Gibis, 2007). Marinades are traditionally used to enhance the sensory qualities of meat (texture, color, flavor) and have been shown to influence PAH formation (Wongmaneeprapit and Vangnai, 2017). Since both HCAs and PAHs are formed through radical-driven reactions, antioxidants can interfere with these processes and inhibit their formation (Lu *et al.*, 2018). For example, phenolic antioxidants have been shown to inhibit the formation of imidazoquinoline-type HCAs via free radical Maillard intermediates (Min *et al.*, 2018). Marinating pork belly with tea has also been shown to increase radical-scavenging activity, preserving meat quality and reducing benzo[a]pyrene formation during grilling (Park *et al.*, 2017).

Garlic, a member of the *Allium* family, is rich in antioxidants, particularly organosulfur compounds like diallyl sulfide (DAS) and diallyl disulfide (DAD) (Mellado-García *et al.*, 2015). These compounds contribute to garlic's aroma and exhibit strong radical-scavenging activity that may inhibit harmful compounds formed during high-temperature cooking. Garlic essential oil has significantly reduced benzo[a]pyrene in charcoal-grilled pork sausages due to its sulfide content (Hu *et al.*, 2021). Garlic marinades also reduce PAH formation by limiting lipid oxidation and protein pyrolysis (Zhang *et al.*, 2022; Lu *et al.*, 2018). In Thai cuisine, garlic is a key marinade ingredient, valued for both flavor and functional health benefits.

The present study aimed to investigate how varying concentrations of garlic in marinades (8%, 15%, and 26%) affect key chemical reactions during grilling, specifically the Maillard reaction and the formation of polycyclic aromatic hydrocarbons (PAHs) in chicken breast grilled on a hotplate. The dependent variables included Maillard browning (color changes and melanoidin absorbance), antioxidant capacity (DPPH, ABTS, and total phenolics), and levels of 16 PAHs (measured by GC-MS). This study explores how the antioxidant properties of garlic in marinades may influence these chemical reactions and potentially reduce the formation of carcinogenic PAHs during grilling, thus contributing to safer and healthier consumption of grilled chicken in Thai cuisine.

2. Materials and Methods

2.1 Preparation of chicken breast sample

Chicken breasts were obtained from Betagro Public Co., Ltd. (Thailand), vacuum-packed, and stored at -20 °C until use. Prior to experimentation, the samples were thawed at 4 °C for 12 hours in a refrigerator to minimize moisture loss and microbial growth. After thawing, any visible fat and connective tissue were trimmed, and the chicken was cut into uniform pieces approximately 3 × 4 × 1 cm (length × width × thickness).

2.2 Preparation of marinade

Before grilling, the chicken breast samples were marinated at a 1:1 weight ratio (meat to marinade) for 12 hours at 4 °C. The marinade base was composed of water, sugar, oyster sauce, and salt. Fresh garlic was added to the base at concentrations of 8%, 15%, and 26% (w/w) for the treatment groups. The control group was included, in which chicken was marinated using the same base formula without garlic.

Fresh garlic bulbs were sourced from a single supplier to ensure batch consistency. Before incorporation, garlic cloves were peeled, washed under running water, and minced using a sanitized food processor. The minced garlic was immediately weighed and mixed into the marinade. All marinades were freshly prepared on the same day of use, and stored at 4 °C prior to marination to maintain ingredient integrity. Preparation and handling were carried out under hygienic conditions to ensure quality control.

2.3 Grilling method

The chicken breast samples were grilled on a preheated hot plate at 350 °C for 5 minutes on each side before analysis, as shown in Figure 1.

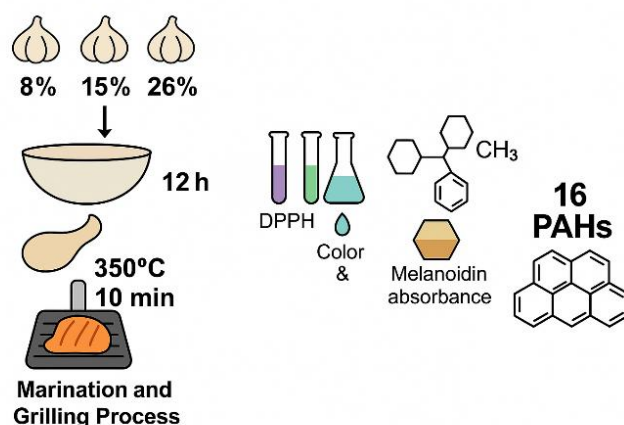


Figure 1 Marination and grilling process.

2.4 Evaluation of antioxidant activity and quantification of phenolic compounds in both marinade and marinated chicken breast

2.4.1 Sample preparation

Marinade and marinated chicken breast samples were ground prior to analysis. The preparation method was adapted from Park *et al.* (2017). Five grams of the ground sample were placed into a stomacher bag containing 45 mL of 0.1 M sodium phosphate buffer (pH 7.0). The buffer was prepared using sodium dihydrogen phosphate (Junsei Chemical, Japan) and disodium hydrogen phosphate (Sigma-Aldrich, USA). The mixture was homogenized for 2 minutes and then filtered through Whatman No. 1 filter paper to remove particulate matter. The filtrate was centrifuged at 12,000 rpm (approximately $14,800 \times g$) for 30 minutes at 4 °C. The resulting supernatant was collected and used for subsequent analysis of antioxidant activity and total phenolic content.

2.4.2 DPPH assay

A volume of 1 mL of 0.2 mM DPPH solution (Sigma Aldrich, USA) prepared in methanol (Merck, Germany) was added to 200 μ L of the sample supernatant and 800 μ L of distilled water in a 12-well plate. The mixture was gently mixed and incubated in the dark for 30 minutes. The control consisted of 1 mL methanol mixed with 1 mL of 0.2 mM DPPH solution, while the blank was 2 mL of methanol. Absorbance was measured at 517 nm using a microplate reader (U-2900UV/VIS Hitachi, Japan) (Park *et al.*, 2017).

2.4.3 ABTS assay

The ABTS solution (Sigma Aldrich, USA) was prepared according to the method described by Serpen *et al.* (2012). The working solution of $\text{ABTS}^{\bullet+}$ was adjusted to an absorbance of 0.75–0.80 at 734 nm. In a 96-well plate, 200 μ L of the $\text{ABTS}^{\bullet+}$ working solution was added to 10 μ L of the sample supernatant and incubated in the dark for 6 minutes. A blank was prepared by mixing 200 μ L of $\text{ABTS}^{\bullet+}$ solution with 10 μ L of buffer. The absorbance of the samples and blank was measured at 734 nm using a microplate reader (U-2900UV/VIS Hitachi, Japan).

The inhibition percentage of the DPPH and $\text{ABTS}^{\bullet+}$ radicals was calculated using the following equation:

$$\% \text{ inhibition}_{\text{sample}} = \frac{[(Abs_{\text{control}} - Abs_{\text{blank}}) - (Abs_{\text{sample}} - Abs_{\text{blank}})]}{Abs_{\text{control}} \times 100}$$

2.4.4 Determination of total phenolic compounds

The total phenolic compound was determined using a modified method based on Tsai *et al.* (2005). Two milliliters of the sample were mixed with 5 mL of 10% Folin-Ciocalteu reagent (Merck, Germany) and allowed to react for 3 minutes. Subsequently, 2 mL of 7.5% (w/v) aqueous sodium carbonate solution (Sigma Aldrich, USA) was added, and the mixture was incubated at room temperature for 1 hour. The absorbance of

the resulting blue color was measured at 765 nm using a microplate reader (U-2900UV/VIS Hitachi, Japan). Total phenolic content was quantified by comparison to a gallic acid standard curve. The total phenolic content (TPC) of the samples was calculated using the following equation:

$$\text{The total phenolic content (TPC)} = \frac{C \times V}{m}$$

where C is the concentration of gallic acid equivalents obtained from the calibration curve (mg/mL), V is the volume of extract (mL), and m is the weight of the sample (g). The results were expressed as milligrams of gallic acid equivalents per gram of sample (mg GAE/g)

2.4.5 pH measurement

The pH (HI5222, USA) of the marinade and marinated chicken breast samples was determined following the method described by Park *et al.* (2017). Five grams of ground sample were homogenized with 45 mL of distilled water for 30 seconds using a blender. The pH of each homogenate was measured at room temperature using a calibrated pH meter. Each sample's pH value was recorded as the average of three independent measurements.

2.5 Non-specific marker of Maillard reaction

2.5.1 Color measurement

Color measurements of grilled chicken were performed using a colorimeter equipped (Color Quest XE, USA) with a D65/8° illumination source, reporting CIELAB values (L^* , a^* , b^*). Measurements were conducted at room temperature ($25 \pm 3^\circ\text{C}$). For each treatment group, three separate samples were evaluated, with color readings taken at four random locations on each sample, avoiding visibly charred areas on the surface.

2.5.2 Absorbance measurement

Melanoidin content was assessed by measuring absorbance at 420 nm, following the method described by Andrade *et al.* (2010). Five grams of the sample were suspended in 10 mL of deionized water, vortexed vigorously for 15 seconds, and then subjected to sonication using an ultrasonic disintegrator for 10 minutes. The mixture was vortexed again for 5 seconds and centrifuged at $14,000 \times g$ for 10 minutes at 4°C. The resulting supernatant was appropriately diluted, and its absorbance was measured at 420 nm using a microplate reader (U-2900UV/VIS Hitachi, Japan). All analyses were conducted in triplicate.

2.6 Determination of PAHs in the sample

2.6.1 Extraction of PAHs

The extraction of PAHs was conducted using a method adapted from Chen and Lin (1997). Chicken samples were ground and freeze-dried, then placed in round filter paper positioned within the center of a Soxhlet extractor. A 500 mL

round-bottom flask containing 200 mL of methanol (Merck, Germany) and 25 mL of 50% aqueous potassium hydroxide (Ajax Finechem, Australia) was attached to the Soxhlet unit for simultaneous PAH extraction and lipid saponification. The mixture was refluxed for 3 hours.

After cooling to 40°C, 150 mL of n-hexane (RCI Labscan, Thailand) was added to the flask with intermittent swirling. The contents were then transferred to a 500 mL separatory funnel containing 150 mL of water. The flask was rinsed twice with 10 mL of methanol, and the rinses were also transferred to the funnel. The funnel was shaken vigorously and left to stand until phase separation occurred. The aqueous layer was discarded, and the remaining aqueous phase was extracted twice more with 150 mL and 100 mL of n-hexane, respectively.

The combined hexane extracts were washed three times with 100 mL of water, dried over anhydrous sodium sulfate (Carlo Erba, Italy), and concentrated to 1 mL using a rotary evaporator. The concentrated extract was applied to a Sep-Pak Florisil cartridge pre-conditioned with 10 mL of methylene chloride (Merck, Germany) and 20 mL of hexane. The cartridge was eluted sequentially with 10 mL of hexane followed by 8 mL of a hexane/methylene chloride mixture (1:1, v/v). The collected eluate was evaporated to dryness, and the residue was reconstituted in 1 mL of methanol/methylene chloride (1:1, v/v).

The final solution was filtered through a 0.2 µm membrane filter and stored in a vial flushed with nitrogen gas until gas chromatography–mass spectrometry (GC-MS) analysis.

2.6.2 PAHs analysis using GC/MS

The extracted samples were analyzed for polycyclic aromatic hydrocarbons (PAHs) using a gas chromatograph (7890A GC System, Agilent Technologies, Canada) equipped with an autosampler and coupled to a mass spectrometer. Separation was achieved using a DB-5MS capillary column (30 m length × 0.25 mm i.d., 0.25 µm film thickness; Agilent Technologies, USA).

A 1 µL aliquot of the sample was injected into the system. The GC oven temperature was initially held at 55°C for 1 minute, then ramped to 320°C at a rate of 25°C/min, and held at 320°C for 3 minutes. Helium served as the carrier gas at a constant flow rate.

The mass spectrometer was operated in electron ionization (EI) mode at 70 eV, with a source temperature of 280°C. Mass spectra were acquired in the range of 45–450 m/z using selective ion monitoring (SIM) mode for increased sensitivity and specificity. Identification and quantification of 16 priority PAHs were performed based on comparison with standard calibration curves, retention times, and characteristic ions, as described in our previous study (Chaemsai *et al.*, 2016). Detailed information on molecular weights and characteristic ions for each PAH is presented in Table 1, while

retention index, retention time, primary, and secondary ions are summarized in Table 2.

2.7 Statistical analysis

All experiments were performed in triplicate, with each sample analyzed three times. Data were expressed as mean ± standard deviation (SD). Statistical analysis was conducted using Minitab 17 (Minitab LLC, USA). One-way ANOVA followed by Fisher's LSD test was used to determine significant differences among groups at $p < 0.05$.

3. Results and Discussion

3.1 Effect of garlic on antioxidant activity and total phenolic content in marinade and marinated chicken breast

The antioxidant activity of marinades and marinated chicken breast samples was assessed using DPPH and ABTS radical-scavenging assays, with results expressed as percentage inhibition. Both assays were used to evaluate the influence of increasing garlic concentrations in the marinade.

As shown in Figure 2, the radical-scavenging activity of the marinade increased with higher garlic content. The marinade containing 26% garlic (marinade 3) exhibited the highest antioxidant activity, followed by those with 15% (marinade 2), 8% (marinade 1), and 0% garlic (control). The percentage inhibition values for both DPPH and ABTS assays were significantly different among treatment groups ($p < 0.05$).

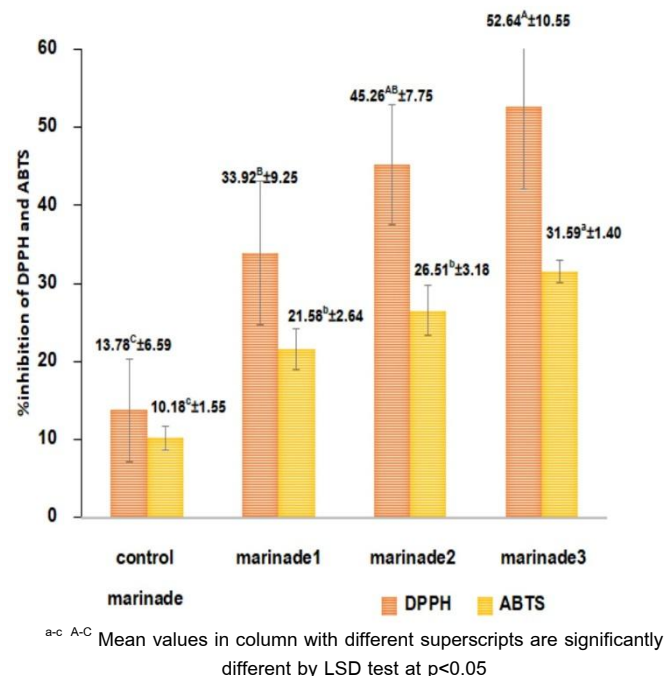
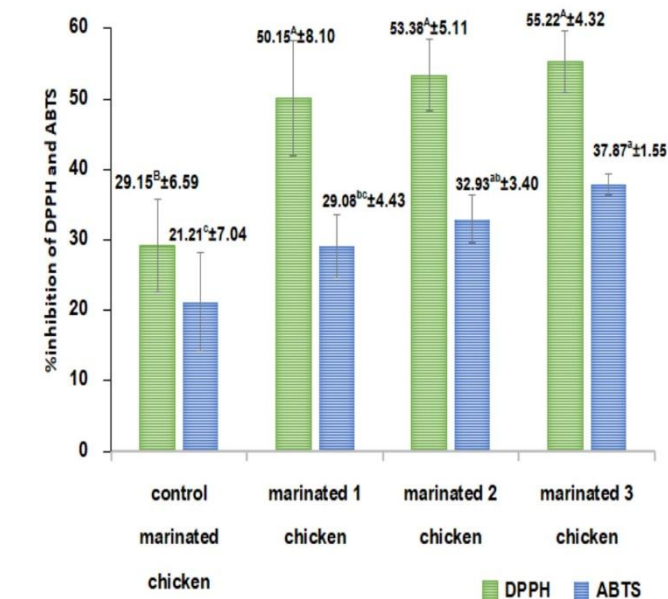


Figure 2 %inhibition of DPPH and ABTS in marinade.

After 12 hours of marination, the DPPH radical-scavenging activity of chicken breast samples was evaluated (Figure 3). The marinated samples showed significantly higher antioxidant activity compared to the control ($p < 0.05$). Although the DPPH activity tended to increase with higher garlic concentrations, there were no statistically significant differences among the

samples marinated with 8%, 15%, and 26% garlic ($p \geq 0.05$). In contrast, ABTS inhibition values differed significantly ($p < 0.05$) among these groups, indicating a garlic concentration-dependent response.

Total phenolic content was quantified using a gallic acid standard curve (0–100 mg/mL), and results were expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g DW). As shown in Figures 4 and 5, total phenolic content in both the marinade and the marinated chicken breast increased proportionally with the garlic concentration. This trend corresponded closely with the observed antioxidant activity in both DPPH and ABTS assays.



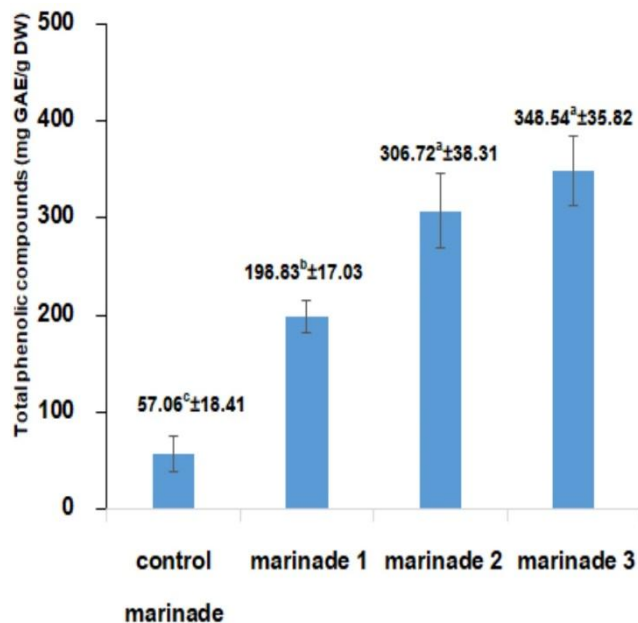
a-c A-C Mean values in column with different superscripts are significantly different by LSD test at $p < 0.05$

Figure 3 %inhibition of DPPH and ABTS in marinated chicken breast.

This divergence may be attributed to the different chemical sensitivities of the two assays. The DPPH assay primarily detects hydrophobic antioxidants in an organic solvent environment and is more sterically hindered, which may limit its interaction with antioxidant compounds in the chicken matrix after grilling. Furthermore, lipophilic compounds from garlic may undergo degradation during cooking or may not be efficiently retained in the meat, reducing their contribution to DPPH radical scavenging. This aligns with findings by Zhang *et al.* (2022), who reported that antioxidant retention post-cooking can be limited by thermal instability and compound solubility.

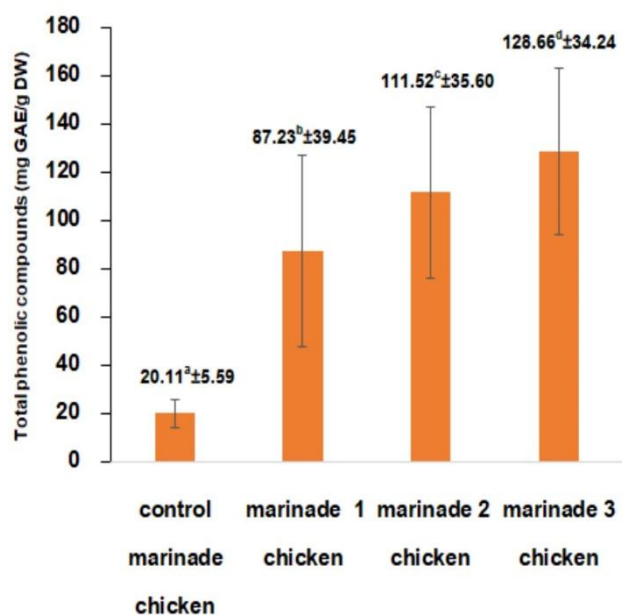
In contrast, the ABTS assay can detect both hydrophilic and lipophilic antioxidants and is generally more responsive to a broader range of phenolic compounds, including those retained in cooked meat. Thus, the increasing trend in ABTS values among marinated samples more accurately reflects the retention of antioxidant capacity post-marination and grilling.

These findings correspond with previous studies such as Mellado-García *et al.* (2015) and Park *et al.* (2017), who found ABTS assays to be more sensitive in detecting phenolic-based antioxidant activity in thermally treated meats.



a-c Mean values in column with different superscripts are significantly different by LSD test at $p < 0.05$

Figure 4 The total phenolic compound of marinade.



a-d Mean values in column with different superscripts are significantly different by LSD test at $p < 0.05$

Figure 5 The total phenolic compound of marinated chicken breast.

Total phenolic content in the marinated chicken breast samples also increased with garlic concentration, supporting the observed antioxidant activity trends. Marinated samples with higher garlic concentrations (marinades 2 and 3) showed significantly greater phenolic content than the control.

The DPPH and ABTS inhibition values observed in this study were relatively low compared to previous findings,

possibly due to the grilling method and duration. Studies such as Lu *et al.* (2018) and Min *et al.* (2018) demonstrated that longer cooking times or more intense heat can degrade antioxidant compounds, lowering measured radical-scavenging activity. In this study, chicken breast samples were grilled for

10 minutes, which was selected to reflect common household cooking practices while minimizing overcooking and sample drying. Extending the grilling time beyond 10 minutes may have resulted in further degradation of antioxidant compounds, leading to even lower DPPH and ABTS values.

Table 1 Characteristic ions and molecular weight for 16 PAHs.

PAHs	Molecular weight	Linear equation
Naphthalene	128.18	$Y = 4.7211 \times 10^5(X) - 7.1305 \times 10^3$
Acenaphthylene	152.20	$Y = 4.3000 \times 10^5(X) - 1.6520 \times 10^4$
Acenaphthene	154.20	$Y = 2.5950 \times 10^5(X) - 4.0760 \times 10^3$
Fluorene	166.23	$Y = 3.1920 \times 10^5(X) - 1.0390 \times 10^4$
Phenanthrene	178.24	$Y = 5.2400 \times 10^5(X) - 1.4540 \times 10^4$
Anthracene	178.24	$Y = 5.2510 \times 10^5(X) - 1.6980 \times 10^4$
Fluoranthene	202.26	$Y = 5.0470 \times 10^5(X) - 1.2670 \times 10^4$
Pyrene	202.26	$Y = 5.5570 \times 10^5(X) - 1.6800 \times 10^4$
Benzo[a]anthracene	228.30	$Y = 4.2980 \times 10^5(X) - 1.2520 \times 10^4$
Chrysene	228.30	$Y = 4.2900 \times 10^5(X) - 1.2590 \times 10^4$
Benzo[b]fluoranthene	252.32	$Y = 5.4470 \times 10^5(X) - 2.2810 \times 10^4$
Benzo[k]fluoranthene	252.32	$Y = 5.4470 \times 10^5(X) - 2.2810 \times 10^4$
Benzo[a]pyrene	252.32	$Y = 4.2110 \times 10^5(X) - 1.8400 \times 10^4$
Benzo[g,h,i]perylene	276.34	$Y = 3.4150 \times 10^5(X) - 1.6680 \times 10^4$
Dibenzo[a,h]anthracene	278.35	$Y = 3.0960 \times 10^5(X) - 1.8380 \times 10^4$
Indeno[1,2,3-c,d]pyrene	276.34	$Y = 3.2440 \times 10^5(X) - 1.8800 \times 10^4$

Y= abundance, X= concentration (ppm)

Table 2 Retention index, retention time, primary and secondary ions of 16 PAHs.

PAHs	Retention index	Retention time (min)	Primary ion	Secondary ion
Naphthalene	1211	5.28	128	129, 127, 51
Acenaphthylene	1370	6.85	152	153, 151, 76
Acenaphthene	1395	7.02	153	154, 152, 76
Fluorene	1450	7.53	166	165, 167, 82
Phenanthrene	1620	8.49	178	176, 179, 76
Anthracene	1630	8.54	178	179, 176, 177
Fluoranthene	1880	9.68	202	203, 200, 201
Pyrene	2150	9.88	202	203, 200, 201
Benzo[a]anthracene	2200	11.11	228	226, 229, 114
Chrysene	2210	11.11	228	226, 229, 114
Benzo[b]fluoranthene	2520	12.05	252	253, 250, 126
Benzo[k]fluoranthene	2530	12.06	252	253, 250, 126
Benzo[a]pyrene	2550	12.44	252	253, 250, 126
Indeno[1,2,3-c,d]pyrene	2790	13.82	276	277, 274, 138
Dibenzo[a,h]anthracene	2800	13.86	278	279, 276, 139
Benzo[g,h,i]perylene	2810	14.20	276	277, 138, 137

3.2 Effect of garlic in marinade on non-specific markers of the maillard reaction in marinated chicken breast

Marination is a widely employed technique in poultry preparation, aimed at enhancing flavor, tenderness, and moisture retention in the final product (Alvarado and McKee, 2007). The composition of the marinade can significantly influence chemical reactions during cooking, particularly the Maillard reaction. While reducing sugars in marinades are known to promote Maillard reaction products, certain spices and acidic ingredients such as onion, lemon juice, and garlic

have been reported to inhibit Maillard reaction products formation (Gibis, 2007).

This study investigated the effect of garlic concentrations in marinades on non-specific markers of the Maillard reaction in grilled chicken breast. Four marinade formulations were examined: a control (no garlic), and three garlic-containing marinades with 8%, 15%, and 26% garlic, respectively. Evaluations included instrumental color analysis and UV–Vis spectrophotometric absorbance at key wavelengths indicative of Maillard reaction progression.

Table 3 Appearance and non-specific indicators of Maillard reaction development in different marinade grilled chicken breast.

Sample	Appearance	Color		
		L* value ^{ns}	a* value ^{ns}	b* value
Control marinated (0% garlic) chicken breast		71.94 ± 1.59	4.81 ± 0.73	21.69 ± 0.54 ^a
Marinated 1 (8% garlic) chicken breast		72.34 ± 1.09	4.49 ± 1.67	22.01 ± 0.73 ^a
Marinated 2 (15% garlic) chicken breast		72.14 ± 1.34	4.55 ± 0.53	21.81 ± 1.27 ^a
Marinated 3 (26% garlic) chicken breast		72.29 ± 1.17	4.26 ± 1.26	19.73 ± 0.49 ^b

Color parameters (L*, a*, and b*) of grilled chicken breast samples were evaluated to assess visual changes associated with the formation of Maillard reaction products. No statistically significant differences ($p \geq 0.05$) were observed in L* (lightness) and a* (redness) values among the different treatments, with L* ranging from 71.94 to 72.34 and a* from 4.26 to 4.81. However, significant differences ($p < 0.05$) were detected in the b* (yellowness) parameter. Specifically, the sample marinated with 26% garlic (marinated 3) exhibited a lower b* value (19.73) compared to the control (0% garlic), 8% garlic (marinated 1), and 15% garlic (marinated 2) samples, which did not differ significantly from each other. These findings suggest that while garlic concentration did not affect lightness or redness, higher garlic levels influenced the yellowness of the grilled chicken breast. Color measurements were performed using a chromameter, with readings taken at three different positions across each chicken breast sample surface to account for non-uniform color distribution. The values were then averaged to provide a more representative color profile. This approach aligns with standard colorimetric practices for

heterogeneous food matrices (Andrade *et al.*, 2010). Sample pH values ranged from 5.89 to 6.03 across all treatments.

To monitor Maillard reaction development, absorbance was measured at 280 nm (early-stage compounds), 360 nm (intermediate products), and 420 nm (melanoidins). Absorbance at 420 nm ranged from 0.16 to 0.48, indicating mild browning in all samples. The b* values further supported these findings, reflecting a yellow hue characteristic of Maillard reaction progression (Andrade *et al.*, 2010).

These results suggest that increasing garlic concentration in the marinade did not significantly alter the color or absorbance properties related to non-specific markers of the Maillard reaction in grilled chicken breast.

3.3 Effect of garlic in marinade on polycyclic aromatic hydrocarbons (PAHs) in marinated chicken breast

Grilled chicken breast samples marinated with four different formulations were analyzed for the presence of 16 priority polycyclic aromatic hydrocarbons (PAHs). According to EU Regulation No. 835/2011, which applies to smoked and heat-treated meat products including those subjected to grilling and

barbecuing benzo[a]pyrene serves as a marker compound for PAH contamination. The regulation stipulates a maximum permissible level of 0.005 µg/g wet weight for benzo[a]pyrene, and a combined maximum of 0.03 µg/g wet weight for benzo[a]pyrene, chrysene, benzo[a]anthracene, and benzo[b]fluoranthene.

Among the grilled chicken breast samples marinated for 12 hours, phenanthrene was detected in the control sample at a concentration of 0.04 µg/g. Phenanthrene is a low-molecular-weight PAH composed of three fused benzene rings and is classified by the International Agency for Research on Cancer (IARC) (International Agency for Research on Cancer, 1993) as group 3, meaning it is not classifiable as to its carcinogenicity to humans.

In the sample marinated with Marinade 3 (containing 26% garlic), benzo[b]fluoranthene was detected at a concentration of 0.006 µg/g. This compound is classified by IARC as group 2B, indicating it is possibly carcinogenic to humans. However, the detected level did not exceed the safety thresholds established by EU Regulation No. 835/2011.

In the present study, increasing concentrations of garlic in the marinade did not result in statistically significant differences in Maillard reaction product formation or polycyclic aromatic hydrocarbon (PAH) levels in grilled chicken breast. Nevertheless, a clear increase in radical-scavenging activity was observed with higher garlic content, indicating an enhancement of antioxidant potential. This suggests that, although Maillard browning and PAH formation remained largely unaffected under the given cooking conditions, the antioxidative components in garlic may still contribute to mitigating oxidative and pyrolytic processes at the molecular level. Recent studies have also demonstrated the antioxidant-rich composition of garlic and its bioactive compounds such as allicin, S-allyl cysteine, and diallyl disulfide capable of modulating oxidative stress and reactive species in thermally processed foods (Batiha *et al.*, 2020; Hossein *et al.*, 2020).

A plausible explanation for the consistently low PAH levels observed across all treatment groups lies in the grilling method employed using a flat hot plate as the heat source. This method likely did not reach the high surface temperatures or direct flame exposure typically necessary to initiate extensive PAH formation. Modern studies support that PAH levels are markedly higher in food subjected to open flame grilling compared to controlled electric or flat-plate systems (Ramos *et al.*, 2019; Malarut and Oonsivilai, 2022). According to Alomirah *et al.* (2011), the occurrence and concentration of PAHs in grilled foods are strongly influenced by parameters such as heat intensity, duration, and cooking method. Similarly, Prathomtong *et al.* (2016) demonstrated that higher-molecular-weight PAHs were predominantly formed at grilling temperatures of 400°C compared to 250°C, underscoring the critical role of temperature. More recently, Alshahrani *et al.*

(2021) observed that flame orientation and grilling method significantly affect PAH distribution in meats, confirming that charcoal grilling and high temperatures pose greater risk. Farhadian *et al.* (2010) also reported that vertical gas flame grilling leads to reduced PAH content, likely due to lower fat-to-flame interaction.

Another contributing factor to the low PAH levels in this study is the inherent leanness of chicken breast. Unlike fattier cuts, chicken breast releases minimal lipid during cooking, reducing the likelihood of fat dripping onto heating elements and undergoing pyrolysis a primary PAH formation route. Recent work by Santonicola *et al.* (2021) confirms that fat content plays a decisive role in PAH generation, particularly when pyrolytic conditions are met. In high-fat meats, drippings combust upon contact with high heat or flames, producing free radicals and PAH intermediates. Therefore, the combination of low-fat meat and moderate cooking conditions likely contributed to the minimal PAH formation observed here.

The biochemical mechanism of PAH formation involves pyrolysis of organic material especially lipids and proteins under high heat, which leads to the release of free radicals. These radicals recombine to form thermally stable, multi-ring aromatic structures characteristic of PAHs (Zelinkova and Wenzl, 2015). As this process is radical-mediated, antioxidant compounds present in the marinade especially those found in garlic may act as chain-breaking inhibitors. Garlic is particularly rich in sulfur-containing compounds such as allicin and S-allyl mercaptocysteine, which are known to exhibit strong radical scavenging, metal-chelating, and antioxidant defense properties (Banerjee *et al.*, 2022). These molecules may suppress radical propagation pathways that contribute to PAH synthesis during grilling, even under thermally intense conditions.

Although this study did not observe significant reductions in PAH levels or Maillard reaction markers with increasing garlic concentration, the enhancement of antioxidant capacity in the marinades suggests a latent protective effect. This may become more pronounced under conditions that more strongly favor PAH formation, such as grilling fatty meats over charcoal or prolonged high-heat exposure. Future studies should investigate garlic's efficacy under such conditions, as well as explore synergistic effects with other antioxidant-rich ingredients in functional marinades (Shahraki *et al.*, 2024). Overall, the findings highlight garlic's potential not only as a flavoring agent but also as a functional additive for mitigating heat-induced toxicants in meat products.

4. Conclusion

This study evaluated the influence of garlic-containing marinades on Maillard reaction products and polycyclic aromatic hydrocarbon (PAH) formation in grilled chicken breast. PAHs were generally undetected across all samples,

with the exception of trace amounts of phenanthrene in the control and benzo[b]fluoranthene in the 26% garlic marinade. All detected levels were within the safety limits established by EU Regulation No 835/2011. Garlic concentration did not significantly influence Maillard reaction indicators or PAH levels under the tested grilling conditions. However, antioxidant activity, as measured by DPPH, ABTS, and total phenolic content, increased with higher garlic concentrations, suggesting a potential inhibitory effect on PAH formation. These findings support the use of garlic marinades to enhance the antioxidant properties of grilled chicken. Further studies are recommended to include sensory evaluations, as high garlic concentrations may negatively affect consumer acceptance.

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6. References

- Alomirah, H., Al-Zenki, S., Al-Hooti, S., Zaghloul, S., Sawaya, W., Ahmed, N. and Kannan, K. 2011. Concentrations and dietary exposure to polycyclic aromatic hydrocarbons (PAHs) from grilled and smoked foods. **Food Control** 22(12): 2028–2035.
- Alshahrani, S.M., Alotibi, R.E., Al-Zahrani, H.S. and Al-Zahrani, S.A. 2021. Influence of grilling methods and flame orientation on PAH levels in meat products. **Journal of Food Composition and Analysis** 97: 103754.
- Alvarado, C.Z. and McKee, S.R. 2007. Marination to improve functional properties and safety of poultry meat. **Journal of Applied Poultry Research** 16(1): 113–120.
- Andrade, C.D., Seiquer, I., Haro, A., Castellano, R. and Navarro, M.P. 2010. Development of the Maillard reaction in foods cooked by different techniques: Intake of Maillard-derived compounds. **Food Chemistry** 122: 145–153.
- Banerjee, S., Panda, A., Das, A. and Dey, S. 2022. Garlic-derived bioactive sulfur compounds: Recent developments in their antioxidant and health-promoting activities. **Current Nutrition & Food Science** 18(2): 182–193.
- Batiha, G.E., Beshbishy, A.M., Wasef, L.G., Elewa, Y.H., Al-Sagan, A.A., Abd El-Hack, M.E., Taha, A.E., Abd-Elhakim, M.Y. and Hetta, H.F. 2020. Chemical constituents and pharmacological activities of garlic (*Allium sativum* L.): A review. **Nutrients** 12(3): 872–890.
- Chaemsai, S., Kunanopparat, T., Srichumpuang, J., Nopharatana, M., Tangduangdee, C. and Siri Wattanayotin, S. 2016. Reduction of the polycyclic aromatic hydrocarbon (PAH) content of charcoal smoke during grilling by charcoal preparation using high carbonisation and a preheating step. **Food Additives and Contaminants: Part A** 33(3): 385–390.
- Chen, B.H., and Lin, Y.S. 1997. Formation of polycyclic aromatic hydrocarbons during processing of duck meat. **Journal of Agricultural and Food Chemistry** 45: 1394–1403.
- Farhadian, A., Jinap, S., Abas, F. and Sakar, Z.I. 2010. Determination of polycyclic aromatic hydrocarbons in grilled meat. **Food Control** 21(5): 606–610.
- Farhadian, A., Jinap, S., Hanifah, H.N. and Zaidul, I.S. 2011. Effects of meat preheating and wrapping on the levels of polycyclic aromatic hydrocarbons in charcoal-grilled meat. **Food Chemistry** 124: 141–146.
- Gibis, M. 2007. Effect of oil marinades with garlic, onion, and lemon juice on the formation of heterocyclic aromatic amines in fried beef patties. **Journal of Agricultural and Food Chemistry** 55: 10240–10247.
- Hossen, M.J., Kim, M.Y., Cho, J.Y. and Kim, J.H. 2020. Therapeutic potential of garlic and its bioactive compounds in age-related neurological disorders. **Aging and Disease** 11(6): 1444–1457.
- Hu, G., Cai, K., Li, Y., Hui, T., Wang, Z., Chen, C., Xu, B. and Zhang, D. (2021). Significant inhibition of garlic essential oil on benzo[a]pyrene formation in charcoal-grilled pork sausages relates to sulfide compounds. **Food Research International** 141: 110127–110137.
- International Agency for Research on Cancer. 1993. Some naturally occurring substances: Food items and constituents, heterocyclic aromatic amines and mycotoxins. **IARC Monographs on the Evaluation of Carcinogenic Risks to Humans** 56. Lyon: IARC.
- Janoszka, B. 2020. PAHs and heterocyclic aromatic amines in meat—Formation, occurrence, and exposure. **Food Chemistry** 325: 126787–126800.
- Lee, J.G., Kim, S.Y., Moon, J.S., Kim, S.H., Kang, D.H. and Yoon, H.J. 2016. Effects of grilling procedure on levels of polycyclic aromatic hydrocarbons in grilled meats. **Food Chemistry** 199: 632–638.
- Lu, F., Kuhnle, K., Gunter, M. and Cheng, Q. 2018. The effect of common spices and meat type on the formation of heterocyclic amines and polycyclic aromatic hydrocarbons in deep-fried meatballs. **Food Control** 92: 399–411.
- Malarut, N. and Oonsivilai, A. 2022. Grilling techniques and their effects on PAH formation in meat: A case study in Thai cuisine. **Environmental Science and Pollution Research** 29: 62390–62398.
- Mellado-García, P., Maisanaba, S., Puerto, M., Llana-Ruiz-Cabello, M., Prieto, A.I., Marcos, R. and Cameán, A.M. 2015. Genotoxicity assessment of propyl thiosulfinate oxide, an organosulfur compound from *Allium* extract,

- intended for food active packaging. **Food and Chemical Toxicology** 86: 365–373.
- Min, S., Patra, J.K. and Shin, H.S. 2018. Factors influencing inhibition of eight polycyclic aromatic hydrocarbons in heated meat model system. **Food Chemistry** 239: 993–1000.
- Ni, Z., Lin, H., Zhang, Y. and Lin, Q. 2021. Formation of PAHs in grilled pork and the effect of different grilling methods. **Meat Science** 181: 108703-108718.
- Park, K.C., Pyo, H.S., Kim, W.S. and Yoon, K.S. 2017. Effects of cooking methods and tea marinades on the formation of benzo[a]pyrene in grilled pork belly (Samgyeopsal). **Meat Science** 129: 1–8.
- Phillips, D.H. 1999. Polycyclic aromatic hydrocarbons in the diet. **Mutation Research** 443: 139–147.
- Prathomtong, W., Suthivarakom, K. and Chaiseri, S. 2016. Effect of grilling temperature and time on the formation of polycyclic aromatic hydrocarbons in chicken meat. **Kasetsart Journal - Natural Science** 50(5): 698–705.
- Ramos, S., Alía, M., Bravo, L. and Goya, L. 2019. Comparative study of PAH formation in meat cooked by various household methods. **Food Additives & Contaminants: Part A** 36(3): 431–440.
- Santonicola, S., Albrizio, S., Murru, N. and Mercogliano, R. 2021. Polycyclic aromatic hydrocarbons (PAHs) in grilled meat: A risk analysis study related to fat content. **Foods** 10(6): 1275-1289.
- Serpen, A., Gökmen, V. and Fogliano, V. 2012. Total antioxidant capacities of raw and cooked meats. **Meat Science** 90: 60–65.
- Shahraki, A.H., Rezapour-Firouzi, S. and Golbabaee, F. 2024. Strategies for mitigation of PAHs in grilled meat products: Recent trends and future prospects. **Trends in Food Science & Technology** 139: 585–594.
- Tsai, T.H., Tsai, P.J., and Ho, S.C. 2005. Antioxidant and anti-inflammatory activities of several commonly used spices. **Journal of Food Science** 70: 93–97.
- Wongmaneeprapit, W. and Vangnai, K. 2017. Effects of oil types and pH on carcinogenic polycyclic aromatic hydrocarbons (PAHs) in grilled chicken. **Food Control** 79(9): 119–125.
- Yurchenko, S. and Mölder, U. 2021. PAH content in grilled meat products under different cooking conditions. **Environmental Science and Pollution Research** 28(6): 7110–7118.
- Zelinkova, Z. and Wenzl, T. 2015. The occurrence of polycyclic aromatic hydrocarbons (PAHs) in food and the dietary exposure of European populations. **Comprehensive Reviews in Food Science and Food Safety** 14(1): 1–17.
- Zhang, Y., Deng, Y. and Li, M. 2022. Effect of antioxidant-rich marinades on PAH reduction in grilled beef. **Journal of Agricultural and Food Chemistry** 70(15): 4567–4574.