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# Effects of Gochujang (Korean Red Pepper Paste) Marinade on Polycyclic Aromatic Hydrocarbon Formation in Charcoal-Grilled Pork Belly

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Abstract Charcoal-grilling is a popular cooking method but causes the formation of polycyclic aromatic hydrocarbons (PAHs), which can be harmful to human health. Gochujang marinade is commonly used for flavoring meats during charcoal-grilling. However, the effects of this marinade on PAHs formation during charcoal-grilling are unclear. Here, we evaluated the effects of Gochujang marinade on the formation of 16 PAHs and inhibition rate of major PAHs (benzo[a]anthracene, benzo[b]fluoranthene, and benzo[a]pyrene) in charcoal-grilled pork belly. Pork belly without marinade (PBW) and marinated with Gochujang (PBG) were stored for 10 days at 9°C under vacuum conditions and then charcoal-grilled to different doneness (internal temperatures of 71°C and 81°C). Among 16 PAHs evaluated in this study, 14 PAHs were detected in charcoalgrilled pork belly, regardless of doneness. PAH formation in charcoal-grilled pork belly was higher at an internal temperature of 81°C than at 71°C (p<0.05). Initially, PBG showed reduced total PAH formation and lower percentages of three major PAHs compared with PBW. Storage increased the inhibitory effects of PBG on the 16 PAHs, and the maximum reduction in total 16 PAHs (63.06%) was observed with moderate cooking (71°C) on day 10 (p<0.05). Moreover, marinade and doneness showed a high interaction with regard to PAH contents in charcoal-grilled pork belly (p<0.05-p<0.0001). Therefore, our findings suggested that marinating pork belly with Gochujang and grilling at 71°C could reduce the formation of 16 PAHs in charcoal-grilled pork belly.

**Keywords** polycyclic aromatic hydrocarbon, barbecue, natural antioxidant, storage, doneness

#### Introduction

The use of barbecues or grilling has become increasingly popular in recent years

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in Korea. High-temperature conditions and the use of charcoal during grilling substantially improve flavor. However, undesirable substances, such as polycyclic aromatic hydrocarbons (PAHs) and heterocyclic aromatic amines, are produced during charcoal-grilling (Wang et al., 2019). PAHs are a large group of persistent organic compounds containing two or more fused aromatic rings (Gong et al., 2018), which are considered potentially carcinogenic in humans because of their genotoxic properties (Mejborn et al., 2019). Although the mechanisms through which PAHs form in meats during grilling are not understood fully, some studies have reported three mechanisms of PAH formation in grilled meats, as follows: (i) the pyrolysis of organic compounds or fats in meat creates a layer on the meat containing PAHs; (ii) the incomplete combustion of coals produces smoke, which attaches to the surface of meat; and (iii) melting fats drip over the burning charcoal, causing the formation of PAHs that return to the meat via smoke (García-Lomillo et al., 2017; Viegas et al., 2014; Wang et al., 2019). Additionally, various factors affect the formation of PAHs in meat, including cooking temperature, time, and fat content (Sahin et al., 2020). In particular, high doneness and fat contents in meat can increase the PAH contents in grilled meats (Kim et al., 2021). Based on concerns regarding the types of PAHs generated in grilled meat, the United States Environmental Protection Agency listed 16 PAHs with carcinogenic and mutagenic effects, namely, naphthalene, acenaphthene, acenaphthylene, fluorene, anthracene, phenanthrene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene, benzo[g,h,i]perylene, and indeno[1,2,3-cd]pyrene (Gong et al., 2018). Although these PAHs are present in charcoal-grilled meat at low levels, frequent consumption of charcoal-grilled meats over a long period of time can be harmful to human health. Therefore, it is important to control the formation of these 16 PAHs in grilled meats.

Various studies have evaluated methods to inhibit PAH formation in meats during cooking (Farhadian et al., 2012; Gibis, 2007; Park et al., 2017). Cooking at lower temperatures and for shorter times can inhibit PAH formation in meat (Farhadian et al., 2012). Additionally, preventing melted fat from meats from dripping onto the heat source reduces benzo[a]pyrene contents in grilled pork belly (Park et al., 2017). In addition, marinating with ingredients with antioxidant properties can reduce PAHs formation during the cooking process (Gibis, 2007). Marinating, a traditional cooking technique, is performed to improve the flavor and tenderness of meat in order to satisfy consumer demand (Fasano et al., 2016). Marinating meats with natural antioxidants, such as polyphenols and sulfhydryl compounds in beer, tea, onion, garlic, and lemon, has been reported to reduce the concentrations of PAHs in meats (Viegas et al., 2014; Wang et al., 2019).

Gochujang, Korean fermented red-pepper paste, is traditionally composed of red pepper, grains (e.g., barley, rice, and/or wheat), and soybean Meju with water (Kwon et al., 2015). Recent studies have reported that Gochujang exhibits bioactivities, including anti-atherosclerotic, anti-obesity, and anticholesterol effects (Kim et al., 2019a; Shin et al., 2016; Yang et al., 2018). These bioactivities are thought to be related to the various biological compounds of Gochujang, including polyphenol compounds Meju and capsaicin derivatives in red pepper (Reyes-Escogido et al., 2011; Yang et al., 2018). Koreans enjoy Gochujang as a seasoning for charcoal-grilled pork belly and typically eat pork belly with dipping in Gochujang or cook pork belly on the grill after marinating with Gochujang (Kwon et al., 2015). According to Chung et al. (2009), Gochujang contained high phenolic acids compared to other phenol contents, which showed greater PAH inhibitory effect than other phenolic compounds, such as flavonoids (Wang et al., 2019). So, Gochujang can be used as effective marinades for inhibiting PAH formation in grilled meat. However, the effects of Gochujang marinade on PAHs formation during charcoal-grilling have not been reported.

Therefore, in this study, we evaluated the effects of Gochujang marinade on inhibition of 16 PAHs in charcoal-grilled pork belly cooked to different levels of doneness.

#### **Materials and Methods**

#### **Materials**

Gochujang (Sunchang Gochujang; Chungjungone, Seoul, Korea) used in this study was composed of brown rice (20.4%), red pepper powder (3%), and red pepper seasoning (red pepper powder [8.3%], sea salt, garlic, and onion) with soybeans, alcohol, yeast powder, starch syrup, brown glutinous rice flour, sea salt, and isomaltooligosaccharide. Folin-Ciocalteu phenol reagent was purchased from Sigma-Aldrich (St. Louis, MO, USA). All solvents and chemicals used for PAH analysis were high-performance liquid chromatography grade. PAH standards (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene, dibenzo[a,h]anthracene, and benzo[g,h,i]perylene) and internal standard (ISTD) solution mix (naphthalene-d<sub>8</sub>, acenaphthene-d<sub>10</sub>, phenanthrene-d<sub>10</sub>, chrysene-d<sub>12</sub>, and perylene-d<sub>12</sub>) were purchased from Sigma-Aldrich. All the other chemicals were of analytical grade.

#### Phenol contents in Gochujang

The phenol content was measured using Folin-Ciocalteu colorimetric assays, as described by Singleton et al. (1999). The Gochujang was diluted in methanol, and the diluted sample (0.5 mL) was mixed with distilled water (5 mL) and Folin-Ciocalteu phenol reagent (0.5 mL). The mixed solution was incubated for 3 min at 25°C. Subsequently, 1 mL of 1 N Na<sub>2</sub>CO<sub>3</sub> was added, and the solution was incubated for 90 min at 25°C in the dark. The absorbance of the reactant was measured at 760 nm using a spectrophotometer (Molecular Devices, San Jose, CA, USA). The standard curve was established using gallic acid, and the results were expressed as mg gallic acid equivalent (GAE)/g.

#### **Antioxidant activities in Gochujang**

#### Oxygen radical absorption capacity (ORAC)

ORAC assays were performed according to the method of Kim et al. (2019b). First, 25  $\mu$ L diluted sample was mixed with 150  $\mu$ L of 80 nM fluorescein and incubated for 15 min at 37°C. After incubation, to generate peroxyl radicals, 25  $\mu$ L of 150 mM 2,2'-azobis (2-amidinopropane) hydrochloride was added. The change in the absorbance of the reactant was recorded every minute at excitation (480 nm) and emission (520 nm) wavelengths, at 37°C for 60 min using a spectrophotometer (Molecular Devices). Trolox (Sigma-Aldrich) was used to generate a standard curve, and the results were expressed as mmol trolox equivalent (TE)/g.

#### 2,2-Azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity

The ABTS radical-scavenging activity was evaluated as described by Kim et al. (2019b). To generate the ABTS+ radical, a 14 mM ABTS+ and a 4.9 mM potassium persulfate solution were mixed (1:1, v/v), and the resulting solution was then reacted at 23±1°C for 12 h in the dark. The stock solution was diluted to reach an absorbance of 0.700±0.002 at 735 nm and 30°C. Next, 50 μL sample was reacted with the ABTS+ radical solution (950 μL) for 30 min at 30°C in the dark. The absorbance of the solution was determined at 735 nm. The results were expressed as mmol TE/g.

#### Ferric reducing antioxidant power (FRAP) activity

FRAP assays were carried out according to the method of Kim et al. (2019b). The FRAP working solution was prepared by

mixing 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-S-triazine in 40 mM HCl, and 20 mM FeCl<sub>3</sub>·6H<sub>2</sub>O solution at a ratio of 10:1:1 (v/v/v), respectively. Each sample (25  $\mu$ L) was reacted with the FRAP working solution (175  $\mu$ L) for 30 min at 37°C in the dark. The absorbance of the reaction solution was determined at 590 nm. The results were expressed as mmol TE/g.

#### 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The DPPH radical scavenging activity was determined following the method of Kim and Jang (2021). Each sample (100  $\mu$ L) was reacted with 0.2 mM DPPH solution (100  $\mu$ L) in a 96-well microplate. The mixtures were incubated at 25°C for 30 min in the dark, and absorbance was measured at 517 nm. The results were expressed as mmol TE/g.

#### Preparation of pork belly without marinade (PBW) and pork belly marinated with Gochujang (PBG)

The preparation of PBW and PBG is detailed in Fig. 1. Frozen pork belly (LYD) and Gochujang were purchased from a local supermarket in Korea. The pork belly was cut into cubes measuring 10 cm (length)×5 cm (width)×0.4 cm (thickness). The pork belly was marinated under the following conditions: 71.94% pork belly, 21.58% Gochujang, and 6.48% water, according to previous sensory evaluation and PAHs analysis (data not shown). PBW and PBG were placed into a polyester bag to ripen under vacuum conditions for 24 h at 5°C. The ripened PBW and PBG were stored under vacuum conditions at 9±2°C for 10 days and then used for analysis on days 0, 5, and 10.

#### Charcoal grilling of pork belly

Approximately 1 kg charcoal was placed into a garden-type grill (55 cm width, 34 cm length, and 14 cm height; Allcook, Korea). After ignition, grilling was performed over the charcoal at 600°C and at a distance of 8 cm from the heat source. According to internal temperature, doneness of grilled pork belly divided into two; the total grilling times were 3.5 min for

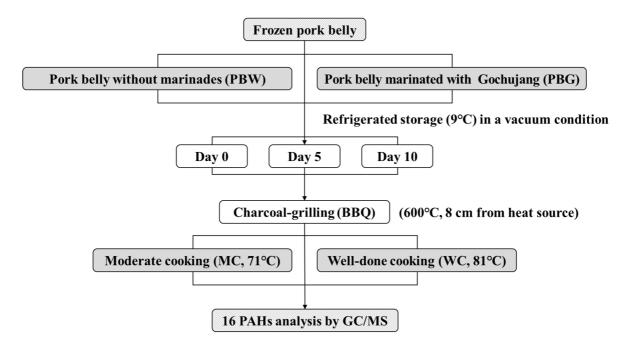


Fig. 1. Diagram of sample preparation procedure.

moderate cooking (internal temperature: 71°C; MC) and 5 min for well-done cooking (internal temperature: 81°C; WC). The internal temperature used in this study fitted on safe temperature for cooking pork meat by USDA guideline (Jang et al., 2019). No oil was applied to the meat surface, and the meat was turned once during cooking.

#### **Analysis of PAHs**

PAH analysis was carried out as described by Kim et al. (2021). Pork belly samples (2.5 g) were weighed in conical tubes, and 5 mL ethyl acetate/acetonitrile (20:80, v/v) with ISTD mix (naphthalene-d<sub>8</sub>, acenaphthene-d<sub>10</sub>, phenanthrene-d<sub>10</sub>, chrysene-d<sub>12</sub>, and perylene-d<sub>12</sub>, 400 ng/mL) was added. The samples were ultrasonicated for 20 min, and after centrifugation (3,000×g, 7 min), the supernatants were transferred to new conical tubes. Next, 5 mL ethyl acetate/acetonitrile (20:80, v/v) was added to the remaining pellets, and samples were ultrasonicated for 20 min. The combined supernatants were concentrated to 2 mL under vacuum conditions using a rotary evaporator (Scilab Korea, Seoul, Korea), and 0.5 mL distilled water was added. The extracts (2 mL concentrate+0.5 mL distilled water, total=2.5 mL) were cleaned up by passing through a Captiva EMR-Lipid cartridge (Agilent Technologies, Santa Clara, CA, USA). Subsequently, 0.625 mL ethyl acetate/acetonitrile/water (16:64:20, v/v/v) was eluted through the Captiva EMR-Lipid cartridge. After elution, 1.875 mL eluent was transferred to a new conical tube, mixed with 2.625 mL distilled water and 1.2 mL isooctane, and shaken vigorously. After centrifugation for 7 min at 3,000×g, PAH contents in the supernatant were analyzed by gas chromatography/mass spectrometry (Agilent 8890 GC with an Agilent 5977B GC/MSD; Agilent Technologies).

The 16 PAHs were separated using a DB-EUPAH capillary column (20 m×0.18 mm id, 0.14 μm thickness; Agilent Technologies). Pure helium (99.999%) was used as the carrier gas at a constant flow rate of 1.2 mL/min. The samples (1 μL) were injected in the spitless mode with an injector temperature of 300°C. The temperature of the mass selective detector was 310°C, and the source temperature was 290°C. The oven temperature was initially held at 70°C for 1 min, ramped to 190°C at a rate of 30°C/min, ramped to 290°C at a rate of 10°C/min and held for 5 min, and then finally ramped to 320°C at a rate of 30°C/min and held for 1 min. The mass spectrometer was operated in electron ionization mode (70 eV), and quantitative data acquisition was performed in selective ion monitoring mode. Representative chromatograms of the 16 PAH standards are shown in Fig. 2. All PAHs were quantified using the relative response factors related to ISTD by nine-point calibration

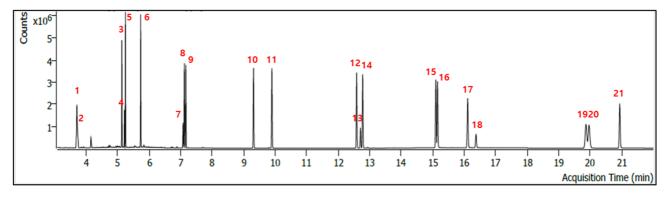


Fig. 2. GC/MS-SIM chromatogram of 16 PAHs standards. 1, Naphthalene-d8 (ISTD); 2, Naphthalene; 3, Acenaphthylene; 4, Acenaphthene-d10 (ISTD); 5, Acenaphthene; 6, Fluorene; 7, Phenanthrene-d10 (ISTD); 8, Phenanthrene; 9, Anthracene; 10, Fluoranthene; 11, Pyrene; 12, Benzo[a]anthracene; 13, Chrysene-d12 (ISTD); 14, Chrysene; 15, Benzo[b]fluoranthene; 16, Benzo[k]fluoranthene; 17, Benzo[a]pyrene; 18, Perylene-d12 (ISTD); 19, Indeno[1,2,3-cd]pyrene; 20, Dibenzo[a,h]anthracene; 21, Benzo[ghi]perylene. PAH, polycyclic aromatic hydrocarbon.

curves (9–2,400 ng/mL). The squared correlation coefficients of determination ( $R^2$ ) of the calibration curves were found to be over 0.99. The limit of detection of 16 PAHs was the range of 0.01–0.09 µg/kg. The limit of quantification of 16 PAHs was the range of 0.03–0.28 µg/kg. The average relative recovery of the 16 PAHs was 80.9%–119.5% for pork belly. Moreover, the relative SD was 0.57%–4.62%.

#### Statistical analysis

All analyses were expressed as means and SEMs. Statistical analysis was performed with SAS software v.9.4 (SAS Institute, Cary, NC, USA) using one-way analysis of variance (ANOVA). The interaction between doneness and marinating with regard to PAH formation in charcoal-grilled pork belly was evaluated using two-way ANOVA by SAS program. Significant differences in means were determined using Tukey's tests and results with p-values less than 0.05 were considered significant.

#### **Results and Discussion**

#### Phenol contents and antioxidant activities of Gochujang

The phenol contents and antioxidant activity of the Gochujang used in this study are shown in Fig. 3. Gochujang had a phenol content of 1.05 mg GAE/g. Red pepper, one of the main components of Gochujang, contains 1.73 mg GAE/g phenols (Marinova et al., 2005). Thus, Gochujang had phenol contents similar to those of broccoli (1.01 mg GAE/g) and apples (1.04 mg GAE/g) (Marinova et al., 2005), but lower phenol contents than green tea (86.3 mg GAE/g dry matter) (Khokhar et al., 2002) and strawberries (2.44 mg GAE/g) (Marinova et al., 2005).

The ORAC activity of Gochujang was 16.62 mmol TE/g, and the ABTS and DPPH radical scavenging activities were 4.30 and 1.77 mmol TE/g, respectively. Additionally, the FRAP activity of Gochujang was 3.24 mmol TE/g. During Gochujang production, various metabolites, such as polyphenols and amino acid, are produced by microbial fermentation; these components have high nutritional value and are important contributors to antioxidant activity in Gochujang (Lee et al., 2016; Yang et al., 2018).

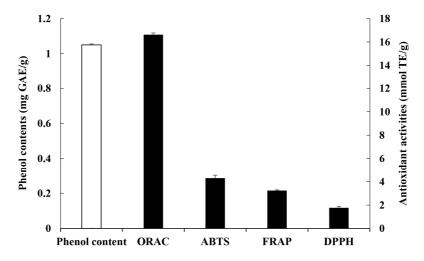


Fig. 3. The phenol content and antioxidant activities of Gochujang.

#### PAH contents in charcoal-grilled pork belly marinated with Gochujang

The contents of 16 PAHs in charcoal-grilled PBW and PBG on days 0, 5, and 10 are shown in Tables 1–3. Several factors affect PAH formation in charcoal-grilled meats, including the amount of fat in the meat, the closeness to the heat source, and the cooking time (Chung et al., 2011). In particular, pyrolysis of proteins, fats, and carbohydrates is accelerated by cooking at high temperature, leading to the generation of PAHs (Kılıç Büyükkurt et al., 2020). In this study, WC increased the PAH contents in PBW and PBG compared with MC (p<0.0001). These results were similar to those of Wang et al. (2019) and Kim et al. (2021), who showed that increased doneness accelerated PAH formation in meats. Phenanthrene was the most abundant compound, with contents ranging from 273.60 to 503.02 μg/kg. Among the four major PAHs detected in this study, benzo[a]pyrene was the most abundant compound, with contents ranging from 16.24 to 63.39 μg/kg. In another study, benzo[a]pyrene was detected in charcoal-grilled pork belly at 8.04 μg/kg (Park et al., 2017), which was lower than the

Table 1. Effect of Gochujang marinade, doneness, and interaction on inhibition of 16 PAHs in charcoal-grilled pork belly on day 0

PAHs (µg/kg)	P	Treat	ment	GEN (	Significance			
	Doneness	PBW	PBG	- SEM	Т	D	T×D	
Naphthalene	MC	141.89 <sup>Aa</sup>	65.94 <sup>Bb</sup>	2.724	****	****	****	
	WC	142.55 <sup>Aa</sup>	111.10 <sup>Ab</sup>	2.024				
	SEM	2.992	1.601					
Acenaphthylene	MC	339.06 <sup>Ba</sup>	290.62 <sup>Bb</sup>	4.196	****	****	****	
	WC	375.81 <sup>Ab</sup>	459.74 <sup>Aa</sup>	4.649				
	SEM	3.891	4.907					
Acenaphthene	MC	11.62 <sup>Aa</sup>	8.71 <sup>Bb</sup>	0.150	****	****	****	
	WC	$10.67^{\mathrm{Ba}}$	12.92 <sup>Aa</sup>	0.173				
	SEM	0.116	0.197					
Fluorene	MC	76.72 <sup>Ba</sup>	62.20 <sup>Bb</sup>	1.323	**	****	****	
	WC	82.30 <sup>Aa</sup>	97.79 <sup>Ab</sup>	0.949				
	SEM	1.174	1.129					
Phenanthrene	MC	417.21 <sup>Ba</sup>	273.60 <sup>Bb</sup>	5.104	****	****	****	
	WC	503.02 <sup>Aa</sup>	473.19 <sup>Ab</sup>	4.779				
	SEM	5.336	4.519					
Anthracene	MC	52.63 <sup>Ba</sup>	34.72 <sup>Bb</sup>	0.478	***	****	****	
	WC	63.83 <sup>Aa</sup>	73.61 <sup>Ab</sup>	0.946				
	SEM	0.493	0.938					
Fluoranthene	MC	162.52 <sup>Ba</sup>	86.35 <sup>Bb</sup>	1.260	****	****	****	
	WC	257.26 <sup>Aa</sup>	217.14 <sup>Ab</sup>	2.002				
	SEM	1.199	2.039					
Pyrene	MC	170.39 <sup>Ba</sup>	91.34 <sup>Bb</sup>	2.258	****	****	****	
	WC	304.42 <sup>Aa</sup>	$260.20^{\mathrm{Ab}}$	2.607				
	SEM	2.149	2.697					

Table 1. Effect of Gochujang marinade, doneness, and interaction on inhibition of 16 PAHs in charcoal-grilled pork belly on day 0 (continued)

DAHa (ug/lsg)	D	Trea	itment	CEM	Sig	gnificance		
PAHs (μg/kg)	Doneness	PBW	PBG	- SEM	T	D	T×D	
Benzo[a]anthracene	MC	$27.82^{\mathrm{Ba}}$	14.28 <sup>Bb</sup>	0.443	****	****	*	
	WC	$50.79^{Aa}$	$40.57^{Ab}$	0.785				
	SEM	0.543	0.720					
Chrysene	MC	ND	ND	-	-	-	-	
	WC	ND	ND	-				
Benzo[b]fluoranthene	MC	18.85 <sup>Ba</sup>	9.34 <sup>Bb</sup>	0.399	****	****	ns	
	WC	35.89 <sup>Aa</sup>	27.17 <sup>Ab</sup>	0.860				
	SEM	0.690	0.650					
Benzo[k]fluoranthene	MC	11.66 <sup>Ba</sup>	6.02 <sup>Bb</sup>	0.260	****	****	ns	
	WC	$23.32^{Aa}$	18.14 <sup>Ab</sup>	0.407				
	SEM	0.356	0.326					
Benzo[a]pyrene	MC	31.73 <sup>Ba</sup>	16.24 <sup>Bb</sup>	0.768	****	****	*	
	WC	63.19 <sup>Aa</sup>	53.51 <sup>Ab</sup>	1.014				
	SEM	0.777	1.007					
Indeno[1,2,3-cd]pyrene	MC	19.87 <sup>Ba</sup>	10.24 <sup>Bb</sup>	0.563	****	****	ns	
	WC	42.13 <sup>Aa</sup>	31.21 <sup>Ab</sup>	0.612				
	SEM	0.594	0.582					
Dibenzo[a,h]anthracene	MC	ND	ND	-	-	-	-	
	WC	ND	ND	-				
Benzo[ghi]perylene	MC	25.25 <sup>Ba</sup>	12.86 <sup>Bb</sup>	0.700	****	****	ns	
	WC	54.26 <sup>Aa</sup>	42.61 <sup>Ab</sup>	0.956				
	SEM	0.631	1.003					
Total 16 PAHs	MC	1,507.22 <sup>Ba</sup>	982.45 <sup>Bb</sup>	18.931	****	****	****	
	WC	$2,009.45^{Aa}$	1,918.90 <sup>Ab</sup>	18.208				
	SEM	19.608	17.477					
4 PAHs	MC	78.40 <sup>Ba</sup>	39.86 <sup>Bb</sup>	1.592	****	****	*	
	WC	149.87 <sup>Aa</sup>	121.26 <sup>Ab</sup>	2.589				
	SEM	1.958	2.325					

 $<sup>^{\</sup>mathrm{A},\mathrm{B}}$  Means within a column with different superscript differ significantly at p<0.05.

amount reported in this study. Notably, the amount of PAH in charcoal-grilled meat increases when the meat is cooked closer to the charcoal; Park et al. (2017) cooked their pork belly at 15 cm from the charcoal, whereas we cooked our pork belly samples at 8 cm from the charcoal. The total PAH contents in PBW-MC and PBW-WC were 1,507.22 and 2,009.45 μg/kg,

 $<sup>^{</sup>a,b}$  Means within a row with different superscript differ significantly at p<0.05.  $^*$  p<0.05,  $^{**}$  p<0.01,  $^{***}$  p<0.001,  $^{****}$  p<0.0001.

<sup>4</sup> PAHs, benzo[a]anthracene+chrysene+benzo[b]fluoranthene+benzo[a]pyrene.

PAH, polycyclic aromatic hydrocarbon; PBW, pork belly without marinade; PBG, pork belly marinated with Gochujang; T, treatment; D, doneness; T×D, treatment×doneness; MC, moderate cooking; WC, well-done cooking; ND, not detected ns, not significant.

respectively. However, PBG reduced the total PAH formation, showing contents of 982.45 and 1,918.90 μg/kg in PBG-MC and PBG-WC, respectively, compared with PBW (p<0.05). Additionally, the contents of the four most abundant PAHs were reduced by PBG (39.86 and 121.26 μg/kg in PBG-MC and PBG-WC, respectively), compared with PBW (p<0.05). Moreover, the contents of 14 PAHs (excluding benzo[b]fluoranthene, benzo[k]fluoranthene, and indono[1,2,3-cd]pyrene) in charcoal-grilled pork belly were affected by treatment (p<0.001, p<0.0001) and doneness (p<0.0001), and treatment and doneness interacted with each other to affect PAH contents (p<0.05, p<0.0001).

On day 5, similar to the results on day 0, 14 PAHs were detected in charcoal-grilled PBW and PBG; chrysene and dibenzo[a,h]anthracene were not detected (Table 2). According to the doneness of charcoal-grilling, WC increased the PAH contents (except the content of naphthalene) in PBW and PBG compared with MC (p<0.0001). Naphthalene contents in charcoal-grilled pork belly were not significantly affected by doneness. Phenanthrene was the most abundant compound, with contents ranging from 319.93 to 751.04 µg/kg. Among the four major PAHs, benzo[a]pyrene was the most abundant, showing contents ranging from 30.62 to 114.84 µg/kg. The total PAH contents for all 16 PAHs in PBW-MC and PBW-WC were 1,919.37 and 3,013.16 µg/kg, respectively; this was increased compared with that from day 0. However, PBG inhibited the formation of total 16PAHs, showing reduced contents of 1,189.63 and 1,402.64 µg/kg for PBG-MC and PBG-WC,

Table 2. Effect of Gochujang marinade, doneness, and interaction on inhibition of 16 PAHs in charcoal-grilled pork belly on day 5

PAHs (μg/kg)	D	Treat	ment	CEM	S	Significance	
	Doneness	PBW	PBG	– SEM	Т	D	$T \times D$
Naphthalene	MC	155.17 <sup>Aa</sup>	47.02 <sup>Ab</sup>	1.293	****	ns	ns
	WC	145.40 <sup>Aa</sup>	49.87 <sup>Ab</sup>	1.656			
	SEM	10.397	0.771				
Acenaphthylene	MC	407.10 <sup>Ba</sup>	287.93 <sup>Ab</sup>	7.074	****	*	**
	WC	453.24 <sup>Aa</sup>	278.56 <sup>Ab</sup>	6.032			
	SEM	7.951	4.817				
Acenaphthene	MC	17.49 <sup>Aa</sup>	11.53 <sup>Ab</sup>	0.248	****	***	**
	WC	$14.61^{Ba}$	$10.87^{\mathrm{Ab}}$	0.356			
	SEM	0.288	0.324				
Fluorene	MC	117.11 <sup>Ba</sup>	83.17 <sup>Ab</sup>	2.090	****	***	**
	WC	138.63 <sup>Aa</sup>	85.69 <sup>Ab</sup>	2.650			
	SEM	3.000	1.546				
Phenanthrene	MC	498.10 <sup>Ba</sup>	319.93 <sup>Bb</sup>	6.542	****	****	****
	WC	751.04 <sup>Aa</sup>	383.87 <sup>Ab</sup>	10.997			
	SEM	11.609	5.381				
Anthracene	MC	80.99 <sup>Ba</sup>	55.00 <sup>Bb</sup>	1.302	****	****	****
	WC	118.90 <sup>Aa</sup>	67.96 <sup>Ab</sup>	1.514			
	SEM	1.589	1.209				
Fluoranthene	MC	205.23 <sup>Ba</sup>	119.91 <sup>Bb</sup>	2.211	****	****	****
	WC	405.03 <sup>Aa</sup>	164.56 <sup>Ab</sup>	6.211			
	SEM	5.344	3.861				

Table 2. Effect of Gochujang marinade, doneness, and interaction on inhibition of 16 PAHs in charcoal-grilled pork belly on day 5 (continued)

PAHs (μg/kg)	D	Trea	tment	CEM	S	Significance		
	Doneness	PBW	PBG	– SEM	T	D	T×D	
Pyrene	MC	234.44 <sup>Ba</sup>	146.87 <sup>Bb</sup>	2.707	****	****	****	
	WC	507.13 <sup>Aa</sup>	191.03 <sup>Ab</sup>	5.063				
	SEM	4.970	2.874					
Benzo[a]anthracene	MC	40.21 <sup>Ba</sup>	24.55 <sup>Bb</sup>	0.873	****	****	****	
	WC	$90.37^{Aa}$	$34.50^{Ab}$	1.135				
	SEM	1.310	0.579					
Chrysene	MC	ND	ND	-	-	-	-	
	WC	ND	ND	-				
Benzo[b]fluoranthene	MC	26.90 <sup>Ba</sup>	15.07 <sup>Bb</sup>	0.480	****	****	****	
	WC	62.92 <sup>Aa</sup>	23.67 <sup>Ab</sup>	0.761				
	SEM	0.788	0.435					
Benzo[k]fluoranthene	MC	17.38 <sup>Ba</sup>	9.35 <sup>Bb</sup>	0.553	****	****	****	
	WC	39.88 <sup>Aa</sup>	13.16 <sup>Ab</sup>	0.604				
	SEM	0.776	0.261					
Benzo[a]pyrene	MC	49.64 <sup>Ba</sup>	30.62Bb	1.188	****	****	****	
	WC	114.84 <sup>Aa</sup>	42.39 <sup>Ab</sup>	1.668				
	SEM	1.795	0.986					
Indeno[1,2,3-cd]pyrene	MC	30.65 <sup>Ba</sup>	16.79 <sup>Bb</sup>	0.509	****	****	****	
	WC	72.57 <sup>Aa</sup>	24.42 <sup>Ab</sup>	0.628				
	SEM	0.612	0.528					
Dibenzo[a,h]anthracene	MC	ND	ND	-	-	-	-	
	WC	ND	ND	-				
Benzo[ghi]perylene	MC	38.98 <sup>Ba</sup>	21.87 <sup>Bb</sup>	0.680	****	****	****	
	WC	98.60 <sup>Aa</sup>	$32.09^{\mathrm{Ab}}$	1.312				
	SEM	1.259	0.775					
Total 16 PAHs	MC	1,919.37 <sup>Ba</sup>	1,189.63 <sup>Bb</sup>	30.488	****	****	****	
	WC	3,013.16 <sup>Aa</sup>	1,402.64 <sup>Ab</sup>	31.898				
	SEM	40.947	16.441					
4 PAHs	MC	116.75 <sup>Ba</sup>	70.24 <sup>Bb</sup>	2.513	****	****	****	
	WC	268.13 <sup>Aa</sup>	100.56 <sup>Ab</sup>	3.458				
	SEM	3.798	1.963					

 $<sup>^{</sup>A,B}$  Means within a column with different superscript differ significantly at p<0.05.

wheats within a row within a row within a row with different superscript differ significantly at p<0.05.

\*p<0.05, \*\*p<0.01, \*\*\*\* p<0.001, \*\*\*\* p<0.0001.

4 PAHs, benzo[a]anthracene+chrysene+benzo[b]fluoranthene+benzo[a]pyrene.
PAH, polycycla aromatic hydrocarbon; PBW, pork belly without marinade; PBG, pork belly marinated with Gochujang; T, treatment; D, doneness;

T×D, treatment×doneness; MC, moderate cooking; WC, well-done cooking; ND, not detected ns, not significant.

respectively, compared with PBW (p<0.05). The contents of the four most abundant PAHs were also reduced in PBG to 70.24 and 100.56  $\mu$ g/kg for PBG-MC and PBG-WC, respectively, compared with PBW (p<0.05). The contents of 14 PAHs (except naphthalene) in charcoal-grilled pork belly were affected by treatment (p<0.0001) and doneness (p<0.05, p<0.0001). An interaction effect was also observed between treatment and doneness for PAH contents (p<0.01, p<0.0001).

On day 10, similar to the results from days 0 and 5, 14 PAHs were detected in charcoal-grilled PBW and PBG; chrysene and dibenzo[a,h]anthracene were not detected (Table 3). WC still increased the contents of all PAHs in PBW and PBG compared with MC (p<0.01, p<0.0001). Phenanthrene was the most abundant compound, with contents ranging from 227.88 to 782.97 µg/kg. Among the four major PAHs, benzo[a]pyrene was the most abundant compound, with contents ranging from 20.49 to 134.75 µg/kg. The contents of the total 16 PAHs in PBW-MC and PBW-WC were 2,254.98 and 3,225.90 µg/kg, respectively, indicating increased contents compared with days 0 and 5. However, PBG inhibited the formation of the total 16 PAHs to 883.07 and 11,540.15 µg/kg for PBG-MC and PBG-WC, respectively, compared with PBW (p<0.05). Additionally, the contents of the four most abundant PAHs were reduced in PBG, showing contents of 49.76 and 107.89 µg/kg for PBG-MC and PBG-WC, respectively, compared with PBW (p<0.05). For 14 types of PAHs, the contents in charcoal-grilled pork belly were affected by treatment (p<0.0001) and doneness (p<0.01, p<0.0001). The interaction effect between treatment

Table 3. Effect of Gochujang marinade, doneness, and interaction on inhibition of 16 PAHs in charcoal-grilled pork belly on day 10

PAHs (μg/kg)	Doneness	Treat	ment	– SEM	Significance		÷	
	Doneness	PBW	PBG	- SEM	T	D	T×D	
Naphthalene	MC	189.78 <sup>Aa</sup>	61.90 <sup>Bb</sup>	4.611	****	**	****	
	WC	$130.70^{\mathrm{Ba}}$	87.95 <sup>Ab</sup>	5.133				
	SEM	5.156	4.585					
Acenaphthylene	MC	536.16 <sup>Aa</sup>	191.23 <sup>Bb</sup>	11.049	****	**	***	
	WC	523.94 <sup>Aa</sup>	285.67 <sup>Ab</sup>	9.870				
	SEM	9.016	11.757					
Acenaphthene	MC	15.97 <sup>Aa</sup>	4.16 <sup>Bb</sup>	0.426	****	***	***	
	WC	15.74 <sup>Aa</sup>	9.19 <sup>Ab</sup>	0.310				
	SEM	0.205	0.486					
Fluorene	MC	109.46 <sup>Ba</sup>	42.58 <sup>Bb</sup>	3.643	****	****	ns	
	WC	147.72 <sup>Aa</sup>	88.45 <sup>Ab</sup>	2.525				
	SEM	3.465	2.764					
Phenanthrene	MC	555.98 <sup>Ba</sup>	227.88 <sup>Bb</sup>	13.132	****	****	ns	
	WC	782.97 <sup>Aa</sup>	425.57 <sup>Ab</sup>	14.611				
	SEM	13.751	14.031					
Anthracene	MC	82.37 <sup>Ba</sup>	21.51 <sup>Bb</sup>	1.487	****	****	***	
	WC	138.47 <sup>Aa</sup>	53.75 <sup>Ab</sup>	2.976				
	SEM	2.993	1.454					
Fluoranthene	MC	246.78 <sup>Ba</sup>	89.47 <sup>Bb</sup>	5.717	****	****	***	
	WC	424.89 <sup>Aa</sup>	181.96 <sup>Ab</sup>	10.186				
	SEM	10.343	5.427					

Table 3. Effect of Gochujang marinade, doneness, and interaction on inhibition of 16 PAHs in charcoal-grilled pork belly on day 10 (continued)

PAHs (μg/kg)	D	Treatment — — — — — — — — — — — — — — — — — — —		– SEM	S	Significance		
	Doneness	PBW	PBG	- SEM	T	D	T×D	
Pyrene	MC	283.32 <sup>Ba</sup>	104.54 <sup>Bb</sup>	6.754	****	***	***	
	WC	524.26 <sup>Aa</sup>	217.93 <sup>Ab</sup>	12.998				
	SEM	13.311	6.114					
Benzo[a]anthracene	MC	45.94 <sup>Ba</sup>	16.96 <sup>Bb</sup>	1.191	****	****	****	
	WC	106.04 <sup>Aa</sup>	$38.33^{Ab}$	2.817				
	SEM	2.870	1.056					
Chrysene	MC	ND	ND	-	-	-	-	
	WC	ND	ND	-				
Benzo[b]fluoranthene	MC	30.65 <sup>Ba</sup>	12.31 <sup>Bb</sup>	0.918	****	****	****	
	WC	70.61 <sup>Aa</sup>	26.17 <sup>Ab</sup>	2.154				
	SEM	2.198	0.809					
Benzo[k]fluoranthene	MC	$20.92^{\mathrm{Ba}}$	8.18 <sup>Bb</sup>	0.768	****	****	****	
	WC	$46.05^{\mathrm{Aa}}$	$17.70^{\mathrm{Ab}}$	1.108				
	SEM	1.197	0.621					
Benzo[a]pyrene	MC	58.37 <sup>Ba</sup>	$20.49^{\mathrm{Bd}}$	1.853	****	****	****	
	WC	134.75 <sup>Aa</sup>	43.39 <sup>Ad</sup>	3.714				
	SEM	3.923	1.356					
Indeno[1,2,3-cd]pyrene	MC	34.88 <sup>Ba</sup>	14.68 <sup>Bb</sup>	1.133	****	****	***	
	WC	$78.25^{Aa}$	28.35 <sup>Ab</sup>	2.733				
	SEM	2.794	0.973					
Dibenzo[a,h]anthracene	MC	ND	ND	-	-	-	-	
	WC	ND	ND	-				
Benzo[ghi]perylene	MC	44.41 <sup>Ba</sup>	17.18 <sup>Bb</sup>	1.601	****	****	****	
	WC	101.50 <sup>Aa</sup>	35.75 <sup>Ab</sup>	3.375				
	SEM	3.537	1.202					
Total 16 PAHs	MC	2,254.98 <sup>Ba</sup>	833.07 <sup>Bb</sup>	48.191	****	****	ns	
	WC	3,225.90 <sup>Aa</sup>	1,540.15 <sup>Ab</sup>	70.014				
	SEM	67.566	51.566					
4 PAHs	MC	134.96 <sup>Ba</sup>	49.76 <sup>Bb</sup>	3.927	****	****	****	
	WC	311.41 <sup>Aa</sup>	107.89 <sup>Ab</sup>	8.657				
	SEM	8.948	3.211					

A,B Means within a column with different superscript differ significantly at p<0.05.
a,b Means within a row with different superscript differ significantly at p<0.05.
p<0.05, \*\* p<0.01, \*\*\*\* p<0.001, \*\*\*\* p<0.0001.
4 PAHs, benzo[a]anthracenet-chrysene-benzo[b]fluoranthene+benzo[a]pyrene.

PAH, polycyclic aromatic hydrocarbon; PBW, pork belly without marinade; PBG, pork belly marinated with Gochujang; T, treatment; D, doneness;

T×D, treatment×doneness; MC, moderate cooking; WC, well-done cooking; ND, not detected. ns, not significant.

and doneness was observed for some of the PAHs and all four of the major PAHs (p<0.001, p<0.0001), but not for fluorene, phenanthrene, and the total 16 PAHs.

Few studies have evaluated the effects of storage on PAH formation in grilled meats. In this study, we found that PAH contents in pork belly were increased as the storage time increased except naphthalene and fluorene, which these contents showed an increase on day 5 and decrease on day 10. Similar to our results, Zhao et al. (2018) reported that the concentration of PAHs in oil increased with storage time and that increases in oxidation and radical formation during storage could be attributed to increased PAH concentrations. Although the reason for the increase in PAH contents during storage and after charcoal-grilling is not fully understood, our findings suggested that increased oxidation in pork belly during storage could contribute to production of high PAH contents in pork belly on days 5 and 10 when charcoal-grilling.

#### Inhibition of PAH content by Gochujang marinade in charcoal-grilled pork belly

The Gochujang marinade showed inhibitory effects on PAH formation in charcoal-grilled pork belly during storage (Tables 1–3). The inhibition rates of the three major PAHs (benzo[a]anthracene, B[a]A; benzo[b]fluoranthene, B[b]F; benzo[a]pyrene, B[a]P) and the 16 total PAHs were calculated in PBG and PBW (Fig. 4). On day 0, the inhibition rates of MC on each of the three major PAHs and on the total 16 PAHs were higher than those of WC (34.82%–50.47% versus 4.51%–24.30%; p<0.05). The highest and lowest PAH inhibitory effects were observed for B[b]F and the total 16 PAHs, respectively. This may be because of the high PAH contents in charcoal-grilled pork belly subjected to WC, results in apparent weakening of the inhibitory effects of Gochujang. However, the inhibition rates of WC on the three major PAHs and the total 16 PAHs on day

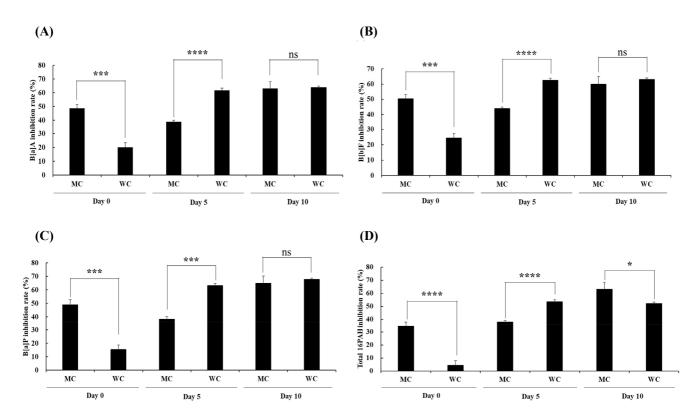


Fig. 4. Percentage of inhibition of major three (A–C) and total 16 PAHs (D) formation in charcoal-grilled marinated pork belly with Gochujang. B[a]A, benzo[a]anthracene; B[b]F, benzo[b]fluoranthene; B[a]P, benzo[a]pyrene. The inhibition rate was calculated by PAHs contents in pork belly marinated with Gochujang compared to that in pork belly without marinade. \* p<0.05, \*\*\* p<0.001, \*\*\*\* p<0.0001. MC, moderate cooking; WC, well-done cooking; ns, not significant; PAH, polycyclic aromatic hydrocarbon.

5 were higher than those of MC (p<0.05). Finally, on day 10, there were no significant differences in inhibition rates between MC and WC for the three major PAHs. Additionally, the inhibition rates of the three major PAHs and the 16 total PAHs increased up to 52.26%–67.80% on day 10. These results indicated that the inhibitory activity of Gochujang on PAH contents in charcoal-grilled pork belly was increased as the storage time increased. Similar to our results, García-Lomillo et al. (2017) reported that red wine pomace seasoning on beef patties for 9 days increased the PAH inhibitory effect compared with that on day 1. This suggests that the inhibitory effects of polyphenols on PAH formation are exerted during storage (García-Lomillo et al., 2017). Therefore, storage of pork belly marinated Gochujang for 10 days could increase the PAH inhibitory effect when charcoal-grilling.

Previous studies have reported that marinades, such as onions, garlic, beer, tea, and vinegar, can reduce the PAH contents of grilled meats (Cordeiro et al., 2020; Viegas et al., 2014; Wang et al., 2019). These ingredients contain abundant amounts of phenolic compounds and sulfhydryl compounds, resulting in high antioxidant activity. In particular, antioxidants can inhibit PAH formation in meats by blocking free radical generation in pyrolysis and eliminating the free radicals (Cordeiro et al., 2020; Viegas et al., 2014; Wang et al., 2019). Gochujang is fermented by *Aspergillus oryzae*, and during fermentation, metabolites such as furan, phenolics, and heptelidic acid derivatives can be produced, exerting various biological effects (Lee et al., 2016). Moreover, during the fermentation, total polyphenols and flavonoids in Gochujang increase, thereby enhancing its antioxidant activity (Yang et al., 2018). Therefore, the high phenolic content and antioxidant activity of Gochujang can inhibit PAH formation in charcoal-grilled pork belly.

The PAH formation during grilling is the result of pyrolysis of nutrients on the surface of meats caused by exposure to high-temperature flame, and incomplete combustion of coal results in adherence of smoke particles to surface of the meat. Therefore, blocking the surface using a marinade can reduce the PAH contents in grilled meats (Kılıç Büyükkurt et al., 2020). Because Gochujang is a paste with high viscosity, it can easily cover the surface of pork belly, which may help block the surface of pork belly from the flame and smoke.

#### **Conclusion**

The phenol contents and antioxidant activities of Gochujang may affect the reduction of PAH contents in charcoal-grilled pork belly. Cooking at a high internal temperature of 81°C (well-done cooking) increased the formation of 16 PAHs in charcoal-grilled pork belly compared with moderate cooking at 71°C. Interactions were observed between doneness and marinating with regard to PAH contents in charcoal-grilled pork belly. Moreover, the inhibitory effects of Gochujang marinade on the total 16 PAHs in charcoal-grilled pork belly increased up to 63.06% after storage for 10 days and cooking at moderate temperature. Importantly, the amount of carcinogenic benzo[a]pyrene in charcoal-grilled PBG was 67.80% on day 10 after well-done cooking. These results indicated that Gochujang marinade of pork belly could inhibit PAH formation after charcoal-grilling and that the inhibitory effects of this marinade increased during the 10 days of storage. These findings provided a preliminary data for the inhibitory effects of Gochujang on PAH formation, while the specific mechanism for PAH inhibitory effect of Gochujang marinade on charcoal-grilled pork belly should be investigated in the future. It was suggested that marinating pork belly with Gochujang may be an effective processing method to reduce the intake of PAHs when consuming charcoal-grilling meat.

#### **Conflicts of Interest**

The authors declare no potential conflicts of interest.

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#### **Author Contributions**

Conceptualization: Park TS, Jin SK, Hur SJ, Lee SK, Jang A. Data curation: Kim HJ, Cho J, Kim D, Park TS, Jin SK, Hur SJ, Lee SK, Jang A. Formal analysis: Kim HJ, Cho J, Kim D. Methodology: Kim HJ, Cho J, Kim D. Software: Kim HJ. Validation: Kim HJ, Kim D, Jang A. Investigation: Cho J, Park TS, Jin SK, Hur SJ. Writing - original draft: Kim HJ, Jang A. Writing - review & editing: Kim HJ, Cho J, Kim D, Park TS, Jin SK, Hur SJ, Lee SK, Jang A.

# **Ethics Approval**

This article does not require IRB/IACUC approval because there are no human and animal participants.

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