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Effect of Beer Marinades on Formation of Polycyclic Aromatic Hydrocarbons in Charcoal-Grilled Pork

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ABSTRACT: The effect of marinating meat with Pilsner beer, nonalcoholic Pilsner beer, and Black beer (coded respectively PB, P0B, and BB) on the formation of polycyclic aromatic hydrocarbons (PAHs) in charcoal-grilled pork was evaluated and compared with the formation of these compounds in unmarinated meat. Antiradical activity of marinades (DPPH assay) was assayed. BB exhibited the strongest scavenging activity (68.0%), followed by P0B (36.5%) and PB (29.5%). Control and marinated meat samples contained the eight PAHs named PAH8 by the EFSA and classified as suitable indicators for carcinogenic potency of PAHs in food. BB showed the highest inhibitory effect in the formation of PAH8 (53%), followed by P0B (25%) and PB (13%). The inhibitory effect of beer marinades on PAH8 increased with the increase of their radical-scavenging activity. BB marinade was the most efficient on reduction of PAH formation, providing a proper mitigation strategy.

KEYWORDS: *polycyclic aromatic hydrocarbons, meat, beer marinades, antiradical activity, food chemistry*

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are found in many common food items.[1] The highest PAH concentration is usually found in smoked and charcoal-grilled products (such as fatty meat and meat products grilled under prolonged or severe conditions), which contribute significantly to the daily intake of these compounds.[2] Even if PAHs are present at low levels, the intake of this type of food can be quite frequent and represent a portion >100 g per meal.[3,4]

PAH accumulation on charcoal-grilled meat results from three possible sources: contamination by smoke generated through the incomplete combustion of the heat source that are brought onto the surface of the food; pyrolysis of organic matter, such as fat, protein, and carbohydrates, directly on the surface of the food; and, mainly, by contact of dripping fat with hot embers.[4−7] The main factors that affect PAH concentrations in charcoal-grilled meat are the closeness to the heat source, the amount of fat in the raw product, and the cooking time.[4,5]

According to the EU Scientific Committee on Food, the most suitable indicator for the occurrence and carcinogenic potency of PAHs in food is the sum of the following eight PAHs (PAH8): benzo(*a*)anthracene (B*a*A), chrysene (Ch), benzo(*b*)fluoranthene (B*b*F), benzo(*k*)fluoranthene (B*k*F), benzo(*a*)pyrene (B*a*P), dibenzo(*a*,*h*)anthracene (D*b*A), benzo(*g*,*h*,*i*)perylene (B*g*P), and indeno(1,2,3-*c*,*d*)pyrene (IP), although it was also concluded that PAH8 do not provide much added value when compared with PAH4 (sum of B*a*A, Ch, B*b*F, and B*a*P).[2] Following this new evidence an EU Commission Regulation established maximum levels for PAH4 in relevant food matrices.[8]

Epidemiological studies correlate the frequent consumption of smoked and grilled meat with high incidence of colorectal cancer.[9] Exposure to PAHs should be as low as reasonably achievable.[10] Thus, mitigation strategies in these types of foods are a matter of concern.

Meat marinating is a popular precooking method in several countries for improving the flavor and tenderness of cooked meat, while reducing the formation of potentially harmful compounds.[11,12] In previous works we demonstrated that marinating meat with beer,[11,12] red[11] or white wine,[12] and tea[13] can be effective strategies for reducing levels of heterocyclic aromatic amines (HAs) in cooked meat, while keeping sensorial acceptance. However, no information is available concerning inhibition of PAHs by using these marinades. Pilsner beer exhibited the best inhibitory effect in HA formation and the better sensorial acceptance when compared with meat marinated with wine and tea.[11−13] Thus, in the present work the effect of different beer marinades (Pilsner beer (PB), nonalcoholic Pilsner beer (P0B), and Black beer (BB)) on PAH8 formation in grilled pork meat (at well-done level) was evaluated. Unmarinated samples cooked in similar conditions provided reference PAH levels. The influence of the antioxidant capacity of beers before and after marinade was also evaluated.

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MATERIALS AND METHODS

Reagents and Standards. The standard PAH mixture in 1 mL of acetonitrile (Supelco, Bellefonte, PA, USA) consisted of 10 μg/mL of naphthalene, acenaphthene, acenaphthylene, fluorene, anthracene, phenanthrene, fluoranthene (F), pyrene, B*a*A, Ch, B*b*F, B*k*F, B*a*P, D*b*A, B*g*P, and IP. PAH standard solution was stored in amber flasks at −20 °C.

Ethanol, methanol, acetonitrile, ethyl acetate, hexane, and dichloromethane were of HPLC grade and were provided by Merck (Darmstadt, Germany). 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Water was purified with a Milli-Q System (Millipore, Bedford, MA, USA). Amber glassware was carefully washed and rinsed with distilled solvent (acetone and hexane) before use.

Marinated Meat Samples and Cooking Conditions. *Preparation of Marinated Meat Samples.* Three different types of beer marinades were tested: Pilsner beer, nonalcoholic Pilsner beer, and Black beer, coded PB, P0B, and BB, respectively. PB contained 5.2% alcohol and was made from water, barley malt, unmalted cereals (maize and barley), and hops; P0B contained 0.5% alcohol and was made from water, barley malt, unmalted cereals (maize), glucose−fructose syrup, flavors, and hops; and BB contained 5.0% alcohol and was made from water, barley malt, sugar, color E150C (ammonia caramel), and hops. All beers were purchased at local supermarkets.

Loin pork steaks were obtained from a local supermarket in Porto, Portugal. Loin pork steaks presented similar dimensions (0.75 cm thick) and weight (about 100 g each). A total of 32 pork loin steaks were used for determination of PAHs: 8 for each marinade and 8 as control samples (unmarinated). Marinade conditions were selected according to previous works:[11,12] marinating time was 4 h at 5 °C, and the proportion of meat amount and marinade volume was 1:1 (g/mL). No other ingredients were added, besides beer. Before cooking, pork samples were removed from the marinade and dried slightly. Four hours of marinating time was selected on the basis of the best beer marinating time for HA inhibition.[11] Recently, Farhadian et al.[14] observed lower PAH concentration in almost all of the analyzed samples with 4 h of marinating.

Cooking Conditions. A bed of charcoal was prepared and ignited in a garden-type grill (35 cm width, 52 cm length, and 15 cm height). When all flames had subsided, pork samples were barbecued at 15 cm distance from the heat source. The grilling temperature was 200−230 °C, and internal temperature reached the minimum 75 °C measured by a digital thermocouple (0560 9260, Testo 926, Lenzkirch, Germany) with a surface probe (0603 1992, Testo 926, Lenzkirch, Germany). Samples were turned once during grilling at half of the total cooking time (10 min). Charcoal was replaced between grilling each type of marinated meat.

After cooking, the eight steaks prepared for each type of marinade were divided in two homogeneous groups of four steaks each. Each group was mixed with a knife mill (Grindomix GM 200, Retsh, Hann, Germany) to obtain a uniform, representative, and homogeneous sample for duplicate analyses. The samples were codified, frozen, and freeze-dried (Cryodos-90, Telstar, Terrassa, Spain). Grilled pork sample codes were as follows: control (unmarinated pork), PBp (pork marinated in Pilsner beer), P0Bp (pork marinated in nonalcoholic Pilsner beer), and BBp (pork marinated in black beer). Average cooking losses were around 54−66 and 50−60% for unmarinated and marinated samples, respectively.

Determination of Marinades Radical-Scavenging in DPPH Reaction. *Extraction of phenolic compounds* of beer marinades was performed according to the method of Zhao et al.[15] and Viegas et al.[12] with some modifications. Marinades were degassed with intensive stirring for 15 min at room temperature. Fifteen milliliters of each sample was extracted during 15 min with 15 mL of ethyl acetate by ultrasound-assisted solvent extraction (FungiLab SA, Barcelona, Spain) and centrifuged at 2000 rpm for 2 min at 20 °C (Eppendorf 5810 R centrifuge, Eppendorf, Hamburg, Germany). The supernatant was collected. Then, 6 g of NaCl was added and the pH was set to 1 (combined pH glass electrode connected to a pH-meter, MicropH 2001, Crison, Barcelona, Spain) using 1 M HCl. The resulting sample was extracted two times more with 15 mL of ethyl acetate. The pooled ethyl acetate extracts were evaporated to dryness under reduced pressure in a rotary evaporator (Rotavapor Büchi RE-111, coupled with a water bath Büchi 461, BÜCHI, Flawil, Switzerland) at 40 °C and redissolved in 10 mL of ethanol 70% (v/v).

Antiradical Activity by DPPH. Extracts of PB, P0B, and BB collected before and after 4 h of marinating were diluted in 96-well microplates (1:2; 1:4; 1:8; 1:16; 1:32; 1:62). A 100 μL amount of 150 μM DPPH and 100 μL of each extract were mixed in the microplates. Absorbances at 517 nm were measured for 2 h until the reaction reached plateau (Biotekmicroplate reader ELX 808, Biotek Corp., USA). For each extract, two readings with DPPH ($A_{extract}$) and one without the radical ($A_{blank\ 1}$) were carried out. DPPH solution were used as control ($A_{control}$), and ethanol 70% was used as blank ($A_{blank\ 2}$). The radical-scavenging activity was expressed as a percentage and calculated by using the following formula:

$$\%\ \text{DPPH scavenging} = 100 - \left(\frac{A_{extract} - A_{blank\ 1}}{A_{control} - A_{blank\ 2}} \times 100 \right)$$

Analysis of PAHs. *Extraction and Cleanup.* PAH extraction and cleanup procedures were performed according to the method of Viegas et al.,[3] with some modifications. Briefly, 2 g of lyophilized meat was weighed into an EPA amber glass vial (40 mL) and was subjected to ultrasound-assisted solvent extraction with 20 mL of *n*-hexane for 1 h at room temperature. The extract obtained was filtered, and the solvent was evaporated nearly to dryness with a rotatory evaporator at room temperature. The residue was redissolved in 3 mL of *n*-hexane and loaded for cleanup in a 5 g silica cartridge (Mega BE-Si, 5 g, 20 mL, from Agilent Technologies) previously washed with 20 mL of dichloromethane, dried completely by vacuum, and conditioned with 20 mL of *n*-hexane. The cartridge was dried by vacuum and eluted with 24 mL of *n*-hexane/dichloromethane (70:30, v/v). The first 5 mL was discharged to reduce the volume of PAH fraction and because no PAHs were detected in this first fraction as described by Viegas et al.,[3] the remaining elution volume was collected in a vial and evaporated to dryness under nitrogen stream at room temperature. The residue obtained was dissolved in 100 μL of acetonitrile.

Chromatographic Conditions. Separation and quantification of PAHs were performed by liquid chromatography with fluorescence detection (HPLC-FLD) according to the method of Viegas et al.[3] A HPLC unit (Jasco, Japan) equipped with a PU-1580 HPLC pump, an AS-950 autosampler with a 20 μL loop, and a FP-920 fluorescence detector was used. PAH separations were performed with a 25 cm × 4.6 mm (length × i.d.), 5 μm particle size, Supelcosil LC-PAH (Supelco, Bellefonte, PA, USA), thermostated at 32.0 ± 0.2 °C. Borwin PDA Controller software (JMBS Developments, Le Fontanil, France) was used. Three solvents were used for mobile phase: 75% methanol in water (A), methanol (B), and ethyl acetate (C) with a flow rate 1 mL/min. The linear gradient program was as follows: 0−18 min, 0−80% B in A; 18−19 min, 80−100% B in A; 19−20 min, 100−90% B in C; 20−28.5 min, 90−82% B in C; 28.5−37.5 min, 82−80% B in C; 37.5−40 min, 80−100% B in C; 40−45 min, 100−0% B in A, followed by rinsing and re-equilibration of the column to the initial conditions. The excitation and emission wavelengths used were 270/390 nm for B*a*A and Ch; 260/430 nm for B*b*F; 290/410 for B*k*F, B*a*P, D*b*A, and B*g*P; and 293/498 for IP. Peak identification in pork samples was carried out by comparing retention times of the peaks with those obtained from a reference standard mixture of PAHs. Quantification of PAHs was performed by standard addition method using nonspiked sample and two fortified levels (10−20 ng/g).[3]

Statistics. The averages of duplicate analyses of two independent experiments were calculated for each PAH. The results were statistically analyzed by analysis of variance. Comparison of mean values was made using the Duncan test. Statistical analyses were all performed with SPSS for Windows version 21 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Antiradical Activities of Beer Marinades. DPPH assay was used to evaluate the antioxidant activity of the marinades under study. The DPPH radical-scavenging activities expressed as percent of inhibition of beer marinades before the addition of meat (T0) and after 4 h of meat marinating (T4) are shown in Figure 1. All beers exhibited radical-scavenging activity. The

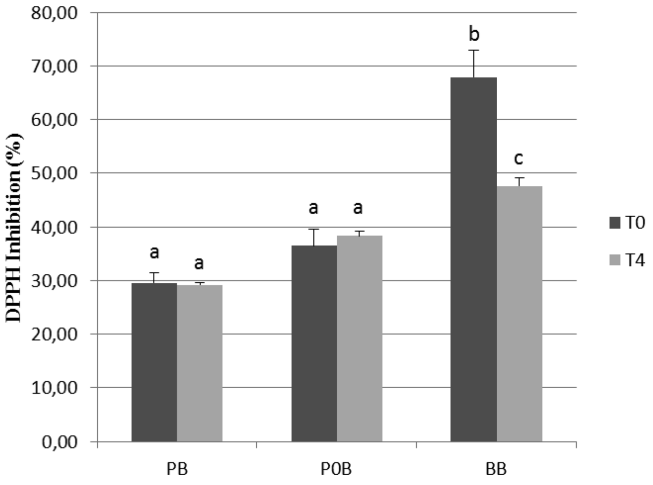

Figure 1. DPPH radical scavenging activity of beer marinades (Pilsner, nonalcoholic Pilsner, and Black beer marinades coded respectively PB, P0B, and BB) before the addition of meat to the marinade (T0) and after 4 h of meat marinating (T4). Results are expressed as percentage of inhibition (%) of DPPH. Error bars represent standard deviation obtained from triplicate experiments. Bars with different letters show significant differences ($p < 0.05$).

results expressed correspond to a 1:8 dilution of beer extracts, necessary to achieve the linearity. As can be seen, at T0, the strongest DPPH-scavenging activity was found in the BB (68.0%), followed by P0B (36.5%) and PB (29.5%). BB exhibited a significantly higher value than others; the slightly higher value of P0B was not significant when compared with PB. These results are in agreement twith those obtained by Tafulo et al.[16] and Polak et al.[17] According to these authors the differences between radical-scavenging activities of the different kinds of beers could be explained by several factors such as the type of fermentation and the presence of food coloring, sweeteners, flavors, and other additives. Ale beers have a higher antioxidant capacity than lager beers.[16] BB is an ale beer in contrast with the rest of the beers studied, which are lager beers. The presence in BB of food coloring (E150C) could be another factor that explains its higher antioxidant activity. In Tafulo et al.,[16] beers with food coloring, including E150C, showed a higher activity antioxidant than ones without it. Nonalcoholic Pilsner beer (P0B) showed a slightly higher antioxidant activity than Pilsner beer (PB). This fact could be explained by the presence in the nonalcoholic Pilsner beer of different additives such as glucose−fructose syrup and flavors. Tafulo et al.[16] also reported that beer samples with sweeteners, flavors, antioxidants, and other additives had a slightly higher antioxidant activity than the samples without it.

After 4 h of meat marinating, no significant changes were observed in radical-scavenging activity of PB and P0B. In our previous work the same behavior was observed for Pilsner beer marinade.[12] However, a significant loss of antiradical capacity was observed in BB after meat marinating. Possible interactions between beer antioxidant compounds and oxidative species of meat surface may occur.

PAH Formation in Charcoal-Grilled Pork. PAH concentrations in charcoal-grilled pork loin are presented in Table 1. All eight PAHs under study were quantified, and their sum was around 21 ng/g wet weight grilled meat. For the quantitative profile, greater contents of Ch > B*a*A > B*bF* > B*a*P were found. B*k*F, D*b*A, B*g*P, and IP were formed in very low concentrations. The four main PAHs formed in charcoal-grilled pork comprise the PAH4. Their contribution to PAH8 was around 80%. Martorell et al.[18] studied 16 PAHs (which include PAH8) in various foodstuffs and also found that lowest PAH levels corresponded to D*b*A, IP, and B*k*F.

Scarce information was found on the individual compounds of PAH4 in grilled meat; several studies describe only B*a*P composition, and few studies mention other PAHs. However, in an EFSA report[2] thousands of food items from several food groups were compared, and the highest mean value was recorded for Ch (3.20 ng/g), followed by B*a*A (1.97 ng/g) and B*b*F (1.48 ng/g); mean contents of these compounds were higher than the mean value for B*a*P (1.02 ng/g). In the present study the PAH4 profile was in agreement with the EFSA statement.

Table 1. PAH8 Formation on Charcoal-Grilled Pork Loin Unmarinated (Control) and Marinated with Pilsner Beer (PBp), Nonalcoholic Pilsner Beer (P0Bp), and Black Beer (BBp)[a]

	PAHs (ng/g wet weight grilled meat)			
PAH	control	PBp	P0Bp	BBp
B*a*A	3.93 ± 0.73 a	3.59 ± 0.90 a	3.55 ± 0.29 a	1.60 ± 0.41 b
Ch	7.45 ± 0.57 a	5.21 ± 2.24 ab	5.79 ± 1.42 ab	3.43 ± 0.49 b
B*b*F	3.23 ± 0.95 a	3.06 ± 0.90 a	2.29 ± 0.24 ab	1.42 ± 0.37 b
B*k*F	0.39 ± 0.32 a	0.61 ± 0.37 a	0.71 ± 0.24 a	0.42 ± 0.17 a
B*a*P	2.71 ± 0.82 a	2.17 ± 0.76 ab	2.03 ± 0.20 ab	1.07 ± 0.08 b
D*b*A	0.24 ± 0.34 a	0.61 ± 0.65 a	0.27 ± 0.16 a	0.44 ± 0.22 a
B*g*P	1.36 ± 0.45 ab	1.80 ± 0.90 b	0.60 ± 0.22 a	0.85 ± 0.13 ab
IP	1.26 ± 0.34 a	0.77 ± 0.57 ab	0.26 ± 0.20 b	0.51 ± 0.13 ab
∑PAH8	20.57	17.82	15.50	9.74

[a]Results are presented as the mean ± standard deviation, $n = 4$. Means with different letters in the same row are significantly different ($p < 0.05$). For all PAHs (except IP) LOD and LOQ were 0.003 and 0.01 ng/g wet weight grilled meat, respectively. LOD and LOQ for IP were 0.01 and 0.04 ng/g wet weight, respectively.

Table 2. Inhibitory Effects of Different Marinating Treatments or Additives on PAH Formation in Cooked Meat

marinating treatment	meat	cooking method	analyzed PAHs	inhibition (%)	ref
basic marinade (sugar, water, onion, turmeric, lemon grass, salt, garlic, coriander, cinnamon)	beef	charcoal-grilled	B*a*P, B*b*F, F	32	14
basic-oil (basic marinade, cooking oil)			B*a*P, B*b*F, F	0.0	
commercial marinade (spices, onion, garlic, salt, sugar, water, cooking oil)			B*a*P, B*b*F, F	9.0	
basic-lemon juice (basic marinade, lemon juice)			B*a*P, B*b*F, F	56	
basic-oil-lemon juice (basic marinade, cooking oil, lemon juice)			B*a*P, B*b*F, F	27	
basic-oil-tamarind (basic marinade, cooking oil, tamarind)			B*a*P, B*b*F, F	0.0	
commercial-tamarind (commercial marinade, tamarind)			B*a*P, B*b*F, F	27	
Pilsner beer	pork	charcoal-grilled	PAH8	13	present work
Pilsner beer without alcohol			PAH8	25	
Black beer			PAH8	53	
marinade (tomato juice, garlic paste, onion, sodium chloride, cumin, coriander, and black pepper)	chicken	microwave oven	U.S. EPA PAHs	89	20
		pan-fried	U.S. EPA PAHs	91	
		direct-flame butane gas	U.S. EPA PAHs	84	
		indirect-flame butane gas	U.S. EPA PAHs	92	
additives					
onion	pork (collars)	pan-fried	F, B*a*A, B*b*F, B*k*F, B*a*P, D*b*A, B*g*P	53	21
	pork (chop)		F, B*a*A, B*b*F, B*k*F, B*a*P, D*b*A, B*g*P	67	
	pork (minced chops)		F, B*a*A, B*b*F, B*k*F, B*a*P, D*b*A, B*g*P	18	
garlic	pork (collars)	pan-fried	F, B*a*A, B*b*F, B*k*F, B*a*P, D*b*A, B*g*P	66	
	pork (chop)		F, B*a*A, B*b*F, B*k*F, B*a*P, D*b*A, B*g*P	41	
	pork (minced chops)		F, B*a*A, B*b*F, B*k*F, B*a*P, D*b*A, B*g*P	0	
spices (cumin, coriander, black pepper, and rosemary)	chicken	microwave oven	U.S. EPA PAHs	56	20
		pan-fried	U.S. EPA PAHs	58	
		direct-flame butane gas	U.S. EPA PAHs	68	
		indirect-flame butane gas	U.S. EPA PAHs	80	
garlic	chicken	microwave oven	U.S. EPA PAHs	76	20
		pan-fried	U.S. EPA PAHs	31	
		direct-flame butane gas	U.S. EPA PAHs	39	
		indirect-flame butane gas	U.S. EPA PAHs	47	
spices and garlic	chicken	microwave oven	U.S. EPA PAHs	88	20
		pan-fried	U.S. EPA PAHs	90	
		direct-flame butane gas	U.S. EPA PAHs	82	
		indirect-flame butane gas	U.S. EPA PAHs	86	

Chung et al.[5] analyzed seven PAHs of the PAH8 in different pork samples and found a total of 10.18 ng/g in charcoal-grilled ones. B*a*P accounted for 2.90 ng/g. Alomirah et al.[19] screened the concentrations of 16 priority PAHs in 72 grilled and smoked food samples and presented mean results; among the PAH8, Ch (4.88 ng/g) and B*a*A (2.27 ng/g) showed the highest mean values, followed by B*b*F (1.58 ng/g) and B*a*P (1.10 ng/g), and the sum of PAH8 was 11.7 ng/g.

Considering the maximum levels of B*a*P (5 ng/g) and PAH4 (30 ng/g) described in European legislation[8] for meat and meat products that have undergone grilling and barbecuing as heating treatment, the values of B*a*P and PAH4 in control samples, 2.71 and 17.32 ng/g, respectively, were below the maximum levels, indicating that they were cooked to a well-done level appropriate for consumption.

Effects of Beer Marinades on PAH Formation. PAH8 concentrations in charcoal-grilled pork loin marinated during 4

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h with different beers are shown in Table 1. As observed for unmarinated meat, PAH8 (B*a*A, Ch, B*b*F, B*k*F, B*a*P, DbA, B*g*P, and IP) were also formed in marinated samples; however, their sum was lower in these samples when compared with control meat. With regard to the three types of beers used as marinades, the less effective in reducing the PAH8 content was PB (17.82 ng/g), followed by P0B (15.50 ng/g). BB had the strongest inhibitory effect on the PAH8 in grilled pork (9.74 ng/g), reducing by more than half the PAH8 content of the control samples. Although not statistically significant, a positive correlation was found between the higher radical-scavenging activity of marinades and a decrease of PAH8 formation (Pearson correlation = 0.606, p = 0.202).

A decrease in antioxidant activity was observed for BB after 4 h; interactions between BB antioxidant compounds and oxidative species of the meat surface may occur, possibly increasing meat resistance to the formation of PAHs.

The exact mechanism of PAH formation is not precisely known; however, it is generally considered that PAHs are formed by condensation of small organic molecules by either pyrolysis or pyrosynthesis.[6,7] At high temperatures these molecules are easily fragmented (pyrolysis) and the free radicals produced recombine to form stable polynuclear aromatic compounds (pyrosynthesis). Because the mechanism of formation of PAHs involves free radicals, it may be possible that antioxidant compounds from beer (especially BB) act as inhibitors in the free radical reaction pathways, through radical quenchers and free radical scavengers activity. Inhibitory effects of different marinating treatments and additives on PAH formation in grilled meat are scarce. Table 2 reports a summary of results from the literature and comparison with the present work. Farhadian et al.[14] evaluated the effect of seven marinade treatments of beef satay on the formation of B*a*P, B*b*F, and F. Inhibitory effects were observed in some marinades; the authors attributed the protective effect due to the presence of spices that are known to have antioxidant activity, emphasizing the sulfur compounds, present in garlic and onion, and their potential to prevent Maillard reactions and then PAH formation. Brady[20] found the highest reduction in samples treated with marinade (from 84 to 92%) followed by spice mixture and garlic paste (from 82 to 90%), spice mixture (from 56 to 80%), and garlic paste (from 31 to 76%) for different cooking methods. However, the charcoal grilling method was not evaluated, and according to EFSA[2] special attention is required for this cooking method because higher PAH content is found in charcoal-grilled products.

Janoszka[21] observed that the addition of onion (30/100 g of meat) and garlic (15/100 g of meat) caused, respectively, average decreases of 60 and 54% of the total content of PAHs (six of the PAH8) in pan-fried pork meat and also connected the effect to the antioxidant effect of polyphenols and sulfhydryl compounds, which can scavenge free radicals and destroy fatty acid hydroperoxides.

In control and marinated samples the PAH8 profiles were quite similar; the predominant PAHs were Ch > B*a*A > B*b*F > B*a*P. However, control samples had greater contents of these PAHs than marinated ones. BB caused a significant decrease these PAHs. B*k*F, D*b*A, B*g*P, and IP were formed in very low concentrations, and no relevant differences between control and marinated were achieved. EFSA[2] concluded that although PAH8 is a suitable indicator of PAHs in food, PAH4 (sum of B*a*P, Ch, B*a*A, and B*b*F) may provide enough information. In fact, the measurement of PAH4 provided a suitable overview of PAH formation and the beer effect on their formation (Figure 2).

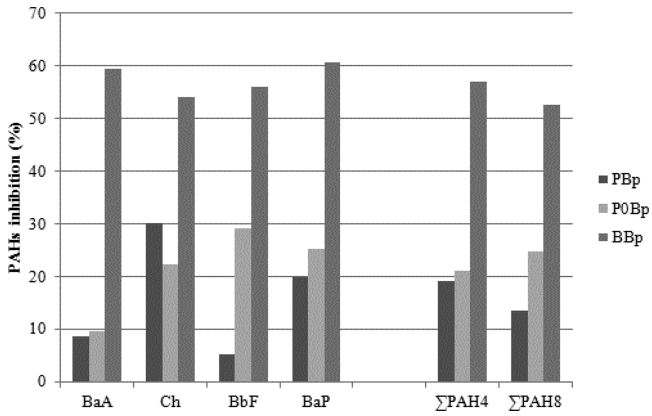

Figure 2. Percentage of inhibition of PAH formation in charcoal-grilled pork loin marinated by different beers (Pilsner, nonalcoholic Pilsner, and Black beer marinades coded respectively PBp, P0Bp, and BBp) versus unmarinated charcoal-grilled loin pork (mean value of quadruplicate analyses). B*a*A, benzo(*a*)anthracene; Ch, chrysene; B*b*F, benzo(*b*)fluoranthene; B*a*P, benzo(*a*)pyrene; ∑PAH4, sum of PAH4; ∑PAH8, sum of PAH8.

With regard to the inhibition of each individual compound, BB showed the highest percentage of inhibition for all with very similar values: 59% of B*a*A, 54% of Ch, 56% of B*b*F, and 61% of B*a*P. P0B and PB presented very similar PAH4 inhibitions, around 20%, but with regard to individual compounds, the inhibitory profile was different. For P0B it was 10% of B*a*A, 22% of Ch, 29% of B*b*F, and 25% of B*a*P. The inhibitory effect of PB was 9% for B*a*A, 30% of Ch, 5% of B*b*F, and 20% of B*a*P.

According to an EFSA report,[2] throughout Europe an exposure of 279 ng/day of PAHs from meat and meat products was estimated on the basis of the occurrence of PAH8 and average consumption of these foods (132 g). Considering an intake of 132 g of grilled pork loin (unmarinated), the uptake of 271 ng of B*a*P and 2057 ng of PAH8 will exceed the overall average dietary exposure of B*a*P (235 ng) and PAH8 (1729 ng) estimated by EFSA. If grilled pork loin marinated in Black beer is consumed, the uptake of 141 ng of B*a*P and 1286 ng in 132 g will not exceed the overall average dietary exposure. Thus, the intake of beer-marinated meat can be a suitable mitigation strategy.

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Author Contributions

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Notes

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