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The Influence of Cooking Methods and Some Marinades on Polycyclic Aromatic Hydrocarbon Formation in Beef Meat

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ABSTRACT

This study aimed to determine the effect of various marinades and their concentrations, as well as various cooking procedures, on polycyclic aromatic hydrocarbon (PAH) formation in cooked beef. The PAH levels in meat were determined, based on the saponification of lipids by methanolic/potassium hydroxide solution, followed by liquid–liquid extraction and QuEChERS method. Pan frying caused lower levels of BaP (1.39 versus 1.62 µg/kg) and PAH4 (5.58 versus 5.73 µg/kg) in beef meat than barbecuing. For sage and thyme extracts prepared at 0.5 to 2.0 °Brix, a significant decrease in the PAH levels of barbecued meat samples was achieved compared to the controls. The levels of PAHs in the samples containing the commercial marinating material were found to be higher than those in the non-marinated control. The BaP and PAH4 levels found in the meat samples marinated with sage and thyme extracts, were below the EU maximum levels. However, BaP was the highest for Control 2 (2.26 µg/kg), and exceeded the EU maximum limit of 2 mg/kg for BaP in heat-treated meat and meat products. The results show a reducing effect of sage and thyme extracts that are normally used during marinating of meat, on the formation of PAH compounds. Consequently, the extracts of sage and thyme, could be used in the commercial marinating material to reduce the level of PAH compounds formed in meat during cooking.

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Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous, carcinogenic pollutants that result from the pyrolysis of organic compounds. The most common PAHs present in the environment contain between two and seven benzene rings. PAHs possessing two or more benzene rings are chemically stable, hydrophobic and lipophilic compounds (1, 2). Light PAHs contain up to four fused benzene rings and heavy PAHs contain more than four benzene rings (3). Heavy PAHs are more stable and toxic than light PAHs (4). Light PAHs are less lipophilic, more volatile and more water-soluble (4).

The major route of dietary exposure to PAHs is through the consumption of food that has been processed at high temperatures, such as via the smoking and grilling of meat and fish products on the

barbecue (2, 5). The amounts of PAH formed in food products, depend on the specific characteristics of the food (particularly the type and content of fat), cooking process (e.g., roasted, grilled, boiled, smoked), type of heat source (e.g., coal, gas, wood, electrical) and the contact and proximity with the heat source (6). Although it has not been conclusively proven, it is thought that PAHs are formed as a result of three types of mechanisms (7). One mechanism is based on the formation of PAHs, by the pyrolysis of organic substances, such as protein, carbohydrate and, in particular, fat, at high temperatures (500–900 °C). Another mechanism is based on the yield from direct contact of lipids dripping at intense heat directly over the flame. This can generate volatile PAHs that, in turn, adhere to the surface of the food, as the smoke rises. The third mechanism is based on the incomplete combustion of coal that forms PAHs, which deposit on the food surface (7, 8).

Despite the existence of more than 200 PAH compounds, the European Union (EU) Scientific Committee on Food has defined only 15 PAH compounds as genotoxic carcinogens. These are benz[a]anthracene (BaA), benzo[b]fluoranthene (BbF), benzo[j]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene (BaP), benzo[g,h,i]perylene, chrysene (Chr), cyclopenta[c,d]pyrene, dibenz[a,h]anthracene, dibenzo[a,e]pyrene, dibenzo[a,h]pyrene, dibenzo[a,i]pyrene, dibenzo[a,l]pyrene, indeno[1,2,3-cd]pyrene and 5-methylchrysene (9). The European Food Safety Authority also identified an additional substance, benzo[c]fluorene, as a priority substance (10). Meat, chicken, fish and their products are among the major food sources that contribute to the daily intake of PAHs by consumers. In 2014, the Commission Regulation No. 1327/2014 established the maximum limit for BaP, to be used as an indicator of carcinogenic PAH formation, as 2.0 µg/kg wet weight and the maximum limit for sum of PAH4 compounds (BaP, BaA, BbF and Chr) as 12 µg/kg wet weight for smoked meat and meat products (11).

Consumers are increasingly relying on convenience food, with barbecued foods becoming more popular, both at home and in restaurants (5, 12). Although these foods contain high amounts of PAHs, among other carcinogenic compounds, the formation of PAHs can be largely prevented during charcoal grilling, when necessary precautions are taken. Grilling at low temperature, avoiding direct contact of meat with the cooking flame, and preventing the dripping of melted oils onto the heat source, can reduce PAH formation (13).

Although the formation of PAHs and other carcinogenic compounds during cooking, such as heterocyclic amines, is strongly influenced by the chemical composition of the food, these can be reduced by addition of specific marinade compositions (13, 14). Generally, the marinating process is applied, to improve the flavor, tenderness and moistness of cooked products (15). Additionally, however, marinating treatment can prevent direct contact between the meat and flame and, thus, also reduce the formation of carcinogenic compounds in barbecued and grilled meat products (16). In particular, various studies have shown that the formation of heterocyclic amines in meat is significantly reduced by treatment with marinades containing antioxidant ingredients (14, 17–20). Among the marinade ingredients that have been used to decrease PAH levels in meat products, are onions and garlic, (21). beer (22). and pickle sauce (vegetable oil, vinegar, paprika, salt and other spices) (23).

Sage (*Salvia officinalis* L.) and thyme (*Thymus vulgaris* L.) are the most important aromatic and medicinal plants of the *Lamiaceae* family. The main phenolic compounds that provide antioxidant properties of sage are carnosol, carnosic acid, rosmadial, rosmanol, epirosmanol and methyl carnosate (24, 25). Besides the phenolic compounds, several other compounds responsible for the antioxidant activity of thyme are thymol, carvacrol, 1.8-cineole, *p*-cymene, linalool, borneol, *a*-pinene and camphor (26). Sage and thyme grow naturally throughout the Mediterranean basin. Sage is consumed as a herbal tea and thyme is widely used as a spice (27, 28). In the literature, there are several studies reporting that sage and thyme are used in meat products to reduce or inhibit lipid oxidation (29, 30). and microbial growth (28, 31, 32). Both of those plants are naturally used in marinating meat products also to provide characteristically flavor, but there is no data about their effects on PAHs formation in meat products, to our knowledge. Therefore, this study aimed to determine the effects of various sage and thyme-based marinades in comparison to commercial marinade and the cooking methods (barbecue and pan-fry) on PAHs formation for each marination in cooked beef.

Table 1. Sample groups used in the research.

Ingredient/ler	Non-marinated Control 1	Marinated with water Control 2	Marinated with different concentrations of sage extract			Marinated with different concentrations of thyme extract		
			S0.5	S1	S2	T0.5	T1	T2
Tenderloin	100 g	100 g	100 g	100 g	100 g	100 g	100 g	100 g
Marinating liquid (29 g)								
–Powder mixture (4 g)	—	29 g/100 g	29 g/100 g	29 g/100 g	29 g/100 g	29 g/100 g	29 g/100 g	29 g/100 g
–Liquid mixture (25 g) (sage or thyme extract:oil, 1:1)	—	Water	0.5 °B sage extract	1 °B sage extract	2 °B sage extract	0.5 °B thyme extract	1 °B thyme extract	2 °B thyme extract

S0.5: meat samples marinated with 0.5 °B sage extract; S1: meat samples marinated with 1 °B sage extract; S2: meat samples marinated with 2 °B sage extract; T0.5: meat samples marinated with 0.5 °B thyme extract; T1: meat samples marinated with 1 °B thyme extract; T2: meat samples marinated with 2 °B thyme extract.

Material and experimental

Preparation of samples

The raw material used in the study was taken as a single piece of bovine carcass from *M. Longissimus dorsi* (*thoracis et lumborum*) muscle, about 10 kg, kept at 4 °C for 24 h prior to marinade. The tenderloin is then divided into 128 equal slices (approximately 10 × 5 × 1 cm, ~60 g), parallel to the longitudinal fiber direction. Different marinade treatments were applied to these slices as following. Slices were randomly divided into two groups for different cooking methods (barbecue and pan fry) including replicate and parallel (32 × 2 × 2). Each of cooking method included eight different marinade treatments (Control 1, Control 2, S0.5, S1, S2, T0.5, T1 and T2). All of the marinade treatments included four slices. The marinating ingredients and process are given in Table 1. The ready-to-use powder marinate mixture, was supplied from a well-known local market and consisted of cornstarch, sugar, salt, onion powder, garlic powder, flavoring, cumin, hot red pepper, red pepper and black pepper. The marinating liquids included the powder and the liquid mixture. These powder and liquid mixtures were added to a container and mixed until a sauce consistency was achieved. The difference in the marinade of the samples was due to the marinating liquid. Instead of water, marinating liquids containing sage and thyme extracts were, respectively, prepared at 0.5, 1.0 and 2.0 °Brix. Sage (*Salvia officinalis* L.) (15 g) and thyme (*Thymus vulgaris* L.) (7.5 g) materials, purchased from the local market as dried herbs packages, were extracted with water (100 ml) in an ultrasonic bath at 40 °C for 30 min. After filtration, the extracts were adjusted to three different concentrations (0.5, 1.0 and 2.0 °Brix) with digital refractometer. These extracts were used in preparing the marinating liquids. In Table 1, meat samples marinated with 0.5, 1.0 and 2.0 °Brix sage extract were represented as S0.5, S1 and S2, respectively. Also, meat samples marinated with 0.5, 1.0 and 2.0 °Brix thyme extract were represented as T0.5, T1 and T2, respectively. Non-marinated meat samples were used as Control 1. Marinated samples, without sage and thyme extracts, were used as Control 2.

The all meat slices, except Control 1, were immersed in the each marinating liquids in separate glass boxes at the same time and aged overnight at 4 °C.

Marinated meat samples were cooked, using two different methods (barbecue and pan fry), until transformed from pinkish-red to brown and the internal temperature of the samples reached 72 °C. For both methods, the meat samples were turned to provide equal cooking on both sides. Cooking took approximately 8 min, slices were turned in every 2 min. The meat samples on the barbecue were 5.5 cm above from the coal and were cooked after the glowing of coal. The cooked meat samples were packed in polyethylene and stored at –20 °C, until analyzed.

General procedure for extraction and cleanup of sample

The samples were extracted, according to a modified method of Larsson et al. (33). Homogenized meat samples (25 g) were weighed into a flask, and 12 g potassium hydroxide and 100 ml ethanol were added

for saponification. Then, 5 ml of 20.5 ppm internal standard anthracene-D₁₀ and 1 ml of 500 ppb PAH mix were added. The mixture was held at 40 °C for 2 h, under a reflux condenser. Next, the solution was filtered, cooled to room temperature, and then transferred to a separating funnel, and mixed with 100 ml water and 100-ml cyclohexane. After the funnel was shaken, and the liquid had separated into layers, the ethanol/water phase was taken up in a separating funnel, and rinsed by adding 50 ml of cyclohexane. The ethanol/water phase was discarded, and the cyclohexane phases were combined.

The cyclohexane phase was washed twice with water (50 ml × 2), methanol/water (4:1, 50 ml) and again twice with water (50 ml × 2). After the cyclohexane extract was rinsed with 50 ml N, N-dimethylformamide/water (9:1), 50 ml of 1% NaCl solution was added and rinsed again. After the formation of the phase separation in the solution, 5 ml of the cyclohexane phase was transferred to QuEChERS dispersive solid phase extractive (dSPE) centrifuge tubes, which contained magnesium sulfate (to remove residual water) and primary, secondary-amine sorbent (to remove sugars and fatty acids), and the cleanup procedure was started. The QuEChERS dSPE tubes were manually shaken for 1 min and then centrifuged at 4000 rpm for 5 min. After centrifugation, 1 ml of the upper phase was transferred to 2-ml vials.

Gas chromatography (GC)-mass spectrometry (MS) analysis

The method developed by Şık et al. (34) for GC-MS analysis, was used to determine the PAHs in the beef samples. The samples, prepared in vials, were injected into the GC system (Thermo Scientific Trace GC Ultra, ISQ Single Quadrupole MS, MA and USA). A Thermo Scientific HP-5MS column (5% phenyl polysilphenylene-siloxane, 15 m × 0.25 mm × 0.25 µm, MA, USA) was used to quantify the PAH compounds. The oven temperature was initially set at 50 °C and held for 0.1 min, then increased to 150 °C at 25 °C/min and held for 1 min, increased to 180 °C at 20 °C/min and held for 1 min, increased to 250 °C at 20 °C/min and held for 1 min, and finally increased to 300 °C at 20 °C/min, which was held for 3.5 min. The carrier gas (helium) was at a constant flow rate of 1 ml/min. The injector and detector temperatures were set at 280 °C.

The PAH-standard mixture and internal standard anthracene-D₁₀, were procured as powders from Dr Ehrenstorfer in high purity (>98%). For the identification of the PAH compounds in the samples, the retention times for each PAH analyte and the major and verifying ions were used to determine the standard mixture (Table 2). The total ion chromatogram is provided in Figure 1. The levels of PAH compounds in the samples were represented as µg/kg wet weight.

Statistical analysis

The experiment involved two types of cooking procedures (barbecued and pan-fried meat), two marinade sources (sage extract and thyme extract), three marinade concentrations (0.5, 1.0 and 2.0 °Brix) and used two control as non-marinated and marinated with water. The marination and cooking procedures were performed in two replicate and parallel. The analysis of the samples was done in parallel. Analysis of variance was performed on the data and Duncan's multiple comparison test was applied to the important

Table 2. Retention times, major and verifying ions of detected PAH compounds.

PAH compounds	Retention time (min)	Major ions	Verifying ions
Anthracene D ₁₀	7.36	188	189, 186
Phenanthrene	7.75	178	179, 176
Fluoranthene	9.76	202	200, 203
Pyrene	10.08	202	200, 203
Benzo[a]anthracene	11.84	228	229, 226
Chrysene	11.91	228	229, 226
Benzo[b]fluoranthene	13.61	252	253, 126
Benzo[a]pyrene	14.00	252	253, 125

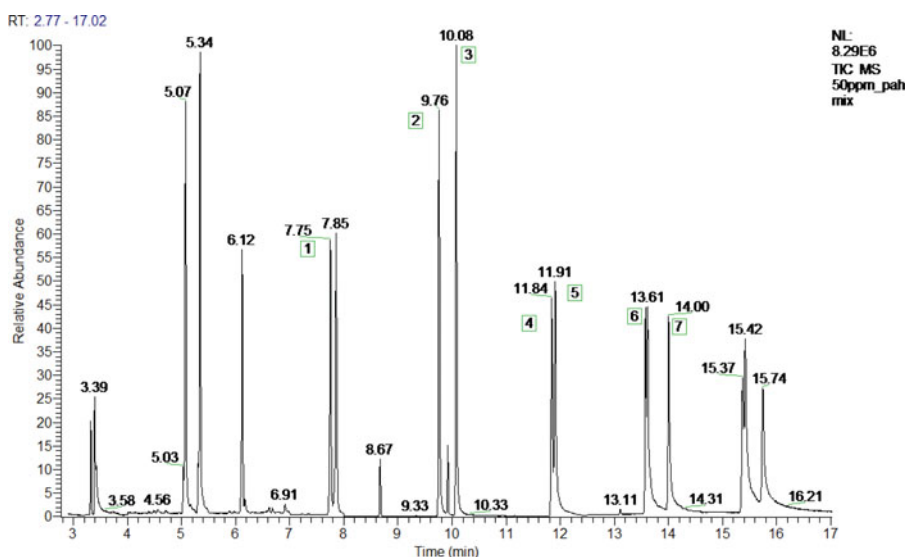


Figure 1. Total ion chromatogram of standards and Phenanthrene (1), Fluoranthene (2), Pyrene (3), Benzo[a]anthracene (4), Chrysene (5), Benzo[b]fluoranthene (6) and Benzo[a]pyrene (7).

factors. All statistical calculations were carried out using SAS software (v.7.00, SAS Institute Inc., Cary, NC, USA). The values are presented as mean \pm standard error.

Result and discussion

The total ion chromatography of the PAH compounds, obtained by injection of the standard mixture solution and the retention time of each PAH compound, is given in Table 2. For the quantification of PAH compounds, the limits of detection and quantification (LOD and LOQ, respectively), calibration curve and coefficients of regression (R^2) are presented in Table 3. The LOQs for selected PAH compounds, were under $2 \mu\text{g}/\text{kg}$ and the linearity of the calibration curves of each PAH compound had a high regression coefficient ($R^2 = 0.99$). Therefore, these results showed sufficient sensitivity for the detection of PAH levels in barbecued and pan-fried meat samples.

The PAH levels of meat samples, for the two different cooking procedures applied, are shown in Table 4. Phenanthrene (Phe), fluoranthene (Fla) and BaP levels were significantly higher in barbecued than pan-fried beef meat. However, statistically comparable pyrene (Pyr), BaA, Chr and PAH4 levels, were found in barbecued and pan-fried beef meat. These results showed that cooking beef meat by pan rather than barbecue may result in lower levels of total PAHs. The PAHs generated from the melting of fat during barbecue cooking, would tend to drip more constantly from beef meat onto the glowing coal directly below the meat. Once formed in the smoke, the PAHs can be deposited onto the surface of the meat. A research by Rose et al. (35) obtained results similar to those presented in the current study.

Table 3. The limits of detection and quantification (LOD and LOQ), calibration curve, coefficients of regression (R^2) of PAH standards obtained by GC-MS.

PAH compounds	LOD ($\mu\text{g}/\text{kg}$)	LOQ ($\mu\text{g}/\text{kg}$)	Calibration curve	R^2	Recovery (%)
Phenanthrene	0.31	1.04	$Y = 0.0344089 + 0.265996 * X$	0.9998	67.91
Fluoranthene	0.39	1.29	$Y = -0.0818268 + 0.216691 * X$	0.9996	71.10
Pyrene	0.43	1.17	$Y = -0.0799653 + 0.234853 * X$	0.9996	82.54
Benzo[a]anthracene	0.41	1.44	$Y = -0.0447536 + 0.0577737 * X$	0.9982	72.20
Chrysene	0.44	1.45	$Y = -0.146796 + 0.23746 * X$	0.9989	75.83
Benzo[b]fluoranthene	0.47	1.51	$Y = -0.2269 + 0.0978053 * X$	0.9966	71.44
Benzo[a]pyrene	0.35	1.18	$Y = -0.0680202 + 0.081999 * X$	0.9979	87.27

Table 4. PAH levels of meat samples applying different cooking method ($\mu\text{g}/\text{kg}$ wet weight).

PAH compounds	Barbecued beef meat	Pan-fried beef meat
Phenanthrene (Phe)	8.57 ^a \pm 0.49	5.64 ^b \pm 0.39
Fluoranthene (Fla)	2.36 ^a \pm 0.17	1.91 ^b \pm 0.11
Pyrene (Pyr)	1.72 ^a \pm 0.13	1.62 ^a \pm 0.07
Benz[a]anthracene (BaA)	2.38 ^a \pm 0.17	2.30 ^a \pm 0.14
Chrysene (Chr)	1.73 ^a \pm 0.10	1.89 ^a \pm 0.08
Benzo[b]fluoranthene (BbFA)	nd	nd
Benzo[a]pyrene (BaP)	1.62 ^a \pm 0.09	1.39 ^b \pm 0.04
PAH4 (BaA, Chr, BbFA, BaP)	5.73 ^a \pm 0.29	5.58 ^a \pm 0.21
Total PAH	18.39 ^a \pm 0.97	14.75 ^b \pm 0.59

nd: not detected.

Pan frying caused lower levels of BaP (1.39 $\mu\text{g}/\text{kg}$) in the samples than barbecuing (1.62 $\mu\text{g}/\text{kg}$). In the literature, smoking (5.34 $\mu\text{g}/\text{kg}$) (36). of beef produced considerably greater BaP levels than grilling (2.74 $\mu\text{g}/\text{kg}$) (36). Similar results were also obtained for chicken meat, where it was reported that the BaP level of pan-fried chicken samples (3.84 $\mu\text{g}/\text{kg}$) was lower than those of direct household butane gas-grilled chicken samples (5.30 $\mu\text{g}/\text{kg}$) (37). However, the BaP levels of boiled meat samples (0.45–1.19 $\mu\text{g}/\text{kg}$) (36). and those cooked in a microwave oven (0.03 $\mu\text{g}/\text{kg}$) (37). were much lower than those in pan-fried meat products.

The PAH levels of barbecued meat samples prepared using the various marinades (water, sage and thyme) are presented in Table 5. Almost all the PAH compounds were significantly affected ($p \leq 0.05$) by the marinade source and concentration. Except for BaA and Chr, the levels of the other PAHs were lower in Control 1 than Control 2. The increased PAH levels may derive from the burning of the marinating powder mixture in Control 2, after water evaporation during cooking.

The meat samples marinated with 2 °Brix sage and thyme extracts were found to contain similar BaP concentrations, although the highest concentration occurred in meat samples marinated with only water (Control 2). The highest PAH4 level was observed in Control 1 (7.49 $\mu\text{g}/\text{kg}$). The lowest level was found in the meat samples marinated with 2 °Brix sage extract (4.15 $\mu\text{g}/\text{kg}$), followed by the meat samples marinated with 2 °Brix thyme extract (4.73 $\mu\text{g}/\text{kg}$). Previously, the levels of BaP and PAH4 were detected at 0.29 and 1.7 $\mu\text{g}/\text{kg}$ in barbecued pork meat, (38). 0.42 and 3.66 $\mu\text{g}/\text{kg}$ in barbecued beef meat (35). and 0.04 and 0.10 $\mu\text{g}/\text{kg}$ in barbecued chicken meat, (39). respectively. In another study, the highest PAH4 level documented was 195 $\mu\text{g}/\text{kg}$, in barbecued pork tenderloin, and the lowest was 0.1 $\mu\text{g}/\text{kg}$, in barbecued chicken breast (40).

The application of sage and thyme extracts, to the surface of the meat, resulted in significantly ($p \leq 0.05$) lower levels of PAHs, possibly due to the antioxidant activity of sage and thyme. It has been reported that antioxidant spices, such as sage and thyme, reduced the formation of carcinogenic compounds in meat products (41). Phenolic compounds contain hydroxyl groups that provide antioxidant properties to the sage and thyme extracts (42). These bioactives can inactivate free radicals, which play an important role in the formation of initial pyrolysis products in the Maillard reaction and, therefore, these antioxidants may reduce the formation of PAH compounds in meat during cooking (13, 43).

The PAH levels of pan-fried meat samples, prepared using the various marinades, are presented in Table 6. Almost all the PAH compounds were significantly affected ($p \leq 0.05$) by the marinade source and concentration. Except for BaA and Chr, the levels of the PAHs in Control 1 were lower than Control 2. These results were similar to those of barbecued meat samples, as above mentioned.

The level of PAHs was slightly lower in pan-fried meat samples marinated with 2 °Brix sage and thyme extracts than the other pan-fried samples. BaP concentrations in all meat samples marinated with the various concentrations of sage and thyme extracts, were comparable, but those of Control 2 were significantly higher ($p \leq 0.05$). Moreover, significantly ($p \leq 0.05$) lower PAH4 levels, 4.66 and 4.72 $\mu\text{g}/\text{kg}$, were found in the meat samples marinated with 2 °Brix sage and thyme extracts, respectively, than the others. It was reported that pan-fried chicken samples treated by marinades, had reduced levels of total PAHs when compared with the non-marinated control sample (37). Similar results were observed in

Table 5. PAH levels of barbecued meat samples prepared using different marinades ($\mu\text{g}/\text{kg}$ wet weight).

PAHs	Control 1	Control 2	S0.5	S1	S2	T0.5	T1	T2
Phe	9.36 ^c \pm 0.10	11.84 ^a \pm 0.03	10.44 ^b \pm 0.43	8.23 ^d \pm 0.02	6.75 ^e \pm 0.01	8.99 ^c \pm 0.05	7.22 ^e \pm 0.03	5.69 ^f \pm 0.04
Fla	2.59 ^b \pm 0.07	3.63 ^a \pm 0.12	2.60 ^b \pm 0.01	2.59 ^b \pm 0.12	2.20 ^c \pm 0.03	2.28 ^c \pm 0.06	1.56 ^d \pm 0.01	1.47 ^d \pm 0.05
Pyr	2.16 ^b \pm 0.07	2.71 ^a \pm 0.06	2.06 ^b \pm 0.02	1.20 ^d \pm 0.06	1.21 ^d \pm 0.02	1.81 ^c \pm 0.07	1.36 ^d \pm 0.03	1.30 ^d \pm 0.00
BaA	3.48 ^a \pm 0.23	2.16 ^{bc} \pm 0.11	2.07 ^{bcd} \pm 0.05	1.90 ^{cd} \pm 0.13	1.63 ^d \pm 0.15	3.34 ^a \pm 0.12	2.43 ^b \pm 0.18	2.06 ^{bcd} \pm 0.02
Chr	2.15 ^{ab} \pm 0.18	1.85 ^{abc} \pm 0.35	1.96 ^{abc} \pm 0.10	1.63 ^{abc} \pm 0.02	1.30 ^c \pm 0.24	2.19 ^a \pm 0.18	1.51 ^{bc} \pm 0.18	1.31 ^c \pm 0.04
BaP	1.87 ^b \pm 0.07	2.26 ^a \pm 0.19	1.95 ^b \pm 0.15	1.33 ^c \pm 0.05	1.22 ^c \pm 0.01	1.48 ^c \pm 0.02	1.44 ^c \pm 0.03	1.37 ^c \pm 0.02
PAH4	7.49 ^a \pm 0.33	6.27 ^b \pm 0.43	5.98 ^{bc} \pm 0.10	4.85 ^d \pm 0.10	4.15 ^e \pm 0.08	7.00 ^a \pm 0.08	5.38 ^{cd} \pm 0.03	4.73 ^{de} \pm 0.08
Total PAH	21.61 ^b \pm 0.29	24.45 ^a \pm 0.59	21.08 ^b \pm 0.32	16.86 ^d \pm 0.05	14.31 ^f \pm 0.05	20.08 ^c \pm 0.01	15.51 ^e \pm 0.02	13.19 ^g \pm 0.01
Mean of total PAH	21.61 ^b \pm 0.29	24.45 ^a \pm 0.59	21.08 ^b \pm 0.32	17.42 ^c \pm 0.07			16.26 ^c \pm 0.00	

S0.5: meat samples marinated with 0.5 °B sage extract; S1: meat samples marinated with 1 °B sage extract; S2: meat samples marinated with 2 °B sage extract; T0.5: meat samples marinated with 0.5 °B thyme extract; T1: meat samples marinated with 1 °B thyme extract; T2: meat samples marinated with 2 °B thyme extract. Results are means \pm standard error.

a,b,c,d,e,f,g Means with different letters within the line indicate differences ($p < 0.05$).

Table 6. PAH levels of pan-fried meat samples prepared using different marinades ($\mu\text{g}/\text{kg}$ wet weight).

PAHs	Control 1	Control 2	S0.5	S1	S2	T0.5	T1	T2
Phe	4.02 ^f \pm 0.00	6.69 ^b \pm 0.10	6.06 ^c \pm 0.06	5.44 ^d \pm 0.02	4.13 ^{ef} \pm 0.01	8.83 ^a \pm 0.12	5.53 ^d \pm 0.21	4.41 ^e \pm 0.07
Fla	2.14 ^b \pm 0.03	2.83 ^a \pm 0.13	1.96 ^{bc} \pm 0.00	1.65 ^{de} \pm 0.02	1.49 ^e \pm 0.03	1.92 ^c \pm 0.06	1.70 ^d \pm 0.03	1.57 ^{de} \pm 0.04
Pyr	1.81 ^b \pm 0.11	2.13 ^a \pm 0.06	1.74 ^{bc} \pm 0.12	1.58 ^{cd} \pm 0.01	1.25 ^e \pm 0.01	1.55 ^{cd} \pm 0.05	1.47 ^d \pm 0.00	1.44 ^{de} \pm 0.00
B[a]A	3.54 ^a \pm 0.19	2.68 ^b \pm 0.25	2.06 ^{bc} \pm 0.22	2.13 ^{bc} \pm 0.29	2.06 ^{bc} \pm 0.18	2.18 ^{bc} \pm 0.00	1.99 ^c \pm 0.12	1.79 ^c \pm 0.07
Chr	2.17 ^a \pm 0.02	2.13 ^a \pm 0.21	2.05 ^{ab} \pm 0.11	1.81 ^{ab} \pm 0.11	1.36 ^c \pm 0.19	2.08 ^{ab} \pm 0.16	1.88 ^{ab} \pm 0.07	1.64 ^{bc} \pm 0.05
B[a]P	1.42 ^b \pm 0.00	1.72 ^a \pm 0.13	1.35 ^b \pm 0.04	1.21 ^b \pm 0.04	1.24 ^b \pm 0.05	1.49 ^{ab} \pm 0.17	1.36 ^b \pm 0.01	1.29 ^b \pm 0.01
PAH4	7.13 ^a \pm 0.16	6.53 ^b \pm 0.09	5.46 ^{cd} \pm 0.16	5.15 ^{def} \pm 0.35	4.66 ^f \pm 0.05	5.75 ^c \pm 0.01	5.23 ^{cde} \pm 0.06	4.72 ^{ef} \pm 0.12
Total PAH	15.10 ^b \pm 0.02	18.18 ^a \pm 0.00	15.23 ^b \pm 0.22	13.82 ^c \pm 0.36	11.52 ^e \pm 0.01	18.05 ^a \pm 0.00	13.93 ^c \pm 0.12	12.13 ^d \pm 0.01
Mean of total PAH	15.10 ^b \pm 0.02	18.18 ^a \pm 0.00	15.23 ^b \pm 0.22	13.52 ^d \pm 0.19	11.52 ^e \pm 0.01	18.05 ^a \pm 0.00	14.71 ^c \pm 0.04	

S 0.5: meat samples marinated with 0.5 °B sage extract; S 1: meat samples marinated with 1 °B sage extract; S 2: meat samples marinated with 2 °B sage extract; T 0.5: meat samples marinated with 0.5 °B thyme extract; T 1: meat samples marinated with 1 °B thyme extract; T 2: meat samples marinated with 2 °B thyme extract. Results are means \pm standard error.

a,b,c,d,e,f Means with different letters within the line indicate differences ($p < 0.05$).

Table 7. Statistical significance and *F* values of the factors and interaction on PAHs content.

PAHs	Cooking method (C)	Marinade treatments (M)	Interaction C × M
Phe	1943.65***	275.69***	100.70***
Fla	197.43***	135.08***	18.83***
Pyr	13.52**	101.18***	16.12***
B[a]A	0.91 ^{ns}	22.44***	5.37**
Chr	3.60 ^{ns}	7.13***	0.52 ^{ns}
B[a]P	27.43***	16.80***	4.29**
PAH4	2.81 ^{ns}	57.86***	4.71**
Total PAH	1161.17***	425.24***	54.08***

ns = not significant.

***p* < 0.01.

****p* < 0.001.

pork meat, pan fried with added onion and garlic and the reduction in the total content of PAHs, was associated with the antioxidant effect of polyphenols and sulfhydryl compounds (21).

The increasing concentration of the sage and thyme extracts, from 0.5 to 2 °Brix, led to a significant decrease in the formation of PAHs of all meat samples compared to Control 1 and 2. The use of these substances in marinade treatments at increasing concentrations has been much more effective (29%) on preventing PAH formation when meat is cooked with barbecue. This may be related to the fact that the antioxidant compounds may have been less damaged in the barbecue cooking process and that the meats were preserved for a longer period of time (44).

Marinade treatments had a significant impact (*p* < 0.001) in PAHs overall levels. Although to a lesser extent, cooking method also had a significant impact (*p* < 0.01) in PAHs at most levels (exception found in BaA and Chr), where Phe and Fla had the higher *F* values (1943.65 and 197.43, respectively) (Table 7). From the evaluated two-way interaction between cooking method and marinade treatments, factors interacted significantly for the majority of analyzed PAHs, apart from Chr, where Phe had the higher *F* value (100.70). In general, in this interaction, the amounts of detected low molecular weight PAHs had high significant values than high molecular weight PAH as BaP, which means cooking method and marination treatments together affects low molecular weight PAHs more than the higher ones. Additionally, from the statistical stand point, while the cooking method was not found to be significantly important on PAH4 levels individually, it was reached to a significant level (*p* < 0.01) as a result of interaction (C × M) effect on the formation of PAH4.

According to European legislation, the maximum permissible levels of BaP and PAH4 in smoked meat products are 2 and 12 µg/kg, respectively (11). Therefore, the BaP and PAH4 levels found in the meat samples marinated with sage and thyme extracts, were below the maximum levels. However, BaP was the highest for Control 2 (2.26 µg/kg), showing that the sample containing only marinating liquid, exceeded the EU maximum limit of 2 mg/kg for BaP in heat-treated meat and meat products. These results indicated that meat products prepared by the cooking and marination procedures presented in the present study are suitable for consumption.

Conclusions

The levels of the PAHs were strongly influenced by the cooking method. Pan frying was a safer cooking method, regarding human exposure to dietary PAHs, than barbecuing. The levels of PAHs in the samples containing the commercial marinating material were found to be higher than those in the non-marinated samples. However, the results show the positive effect of sage and thyme extracts, which are normally used in marinating meat for the improvement of taste, on the formation of PAH compounds, when they are added to commercial marinating material. The levels of PAHs could be significantly reduced, when plant extracts, such as sage and thyme, are added to marinating fluid at increasing concentrations. Consequently, the extracts of sage and thyme, could be used in the commercial marinating material to reduce the level of PAH compounds in addition to their taste affects formed in meat during cooking.

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