Inhibitory Effect of Different Marinade Receipts on the Levels of Polycyclic Aromatic Hydrocarbons Formation in Charcoal Grilled Whole Leg Quarters Chicken

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Abstract:
This study was conducted to assess the inhibitory effects of various marinade receipts on the levels of polycyclic aromatic hydrocarbons PAH4 and PAH8 formation in charcoal grille whole leg quarters chicken. The both PAHs were considered by the International Agency for Research on Cancer ***(IRAC, 2010)*** as carcinogenetic indicators. The mean ±SD values of PAH in control and experiments 1, 2, 3 and4 for PAH4 (benzo [a] anthracene; chrysene; benzo [b] fluoranthene; and benzo [a] pyrene) were 3.45 ± 043**,** 0.25±0.04**,** 0.12±0.01**,** 0.29±0.11 and 0.08±0.00 µg/ kg $^{-1}$respectively. While, for PAH8 (benzo [a] anthracene; chrysene; benzo [b] fluoranthene; benzo [k] fluoranthene, benzo [a] pyrene, Indeno(1,2,3c-d)pyrene, dibenzo(a,h)anthracene and benzo(g,h,i)perylene) were 3.60 ±0.43, 0.34± 0.04 , 0.19 ±0.01**,** 0.90±0.47 and 0.15±0.00 µg/ kg^{-1}respectively in charcoal grille whole leg quarters chicken. Both PAHs were considered by ***IRAC (2010)*** as carcinogenic compounds and PAH4 and PAH8 either individually or in a combination, are currently the only possible indicators of the carcinogenic potency of PAHs in food more than individual benzo (a) pyren ***(EFSA, 2008).*** The inhibitory % of application of different marinade receipts in experiments 1, 2, 3 and 4 were 92.8%, 96.5%, 91.6% and 97.7%; 90.5%, 94.7%, 75.0% and 95.8%, for PAH4 & PAH8 respectively. The results showed that the experiment 4 had the most significant reduction (P = 0.001**) effect for both PAH4 & PAH8 in the experimented charcoal grilled chicken due to the ripening and tenderizing effect of the different marination components on poultry meat and there is a good relation between time and temperature during charcoal grilling on the formation of both PAH4 and PAH8 in grilled chicken whole leg quarters

Keywords: PAH4, PAH8, charcoal Grilled chicken, Marinades.

Introduction

The main purpose of preparation of food is to produce safe food with optimal minimal content of possibly harmful substances. However, cooking and food processing at high thermal treatment have been shown to generate various kinds of cooking toxicants. Polycyclic Aromatic Hydrocarbons (PAHs) are considered one of the cooking toxicants and carcinogenic agents ***(IRAC, 2010 and Viegas et al., 2012a, b),*** and had a great concern about their hazard impact on human health ***(Darwish et al., 2019).*** PAHs are group of more than hundred aromatic chemical compounds which are formed due to the incomplete combustion of organic materials ***(Rengarajan et al., 2015).*** Human exposure varies among individuals to these food toxicants due to dietary habits and cooking practice ***(Purcaro et al., 2009; Viegas et al., 2012a, b).*** Charcoal grilling processes involving either direct or indirect contact with high temperatures are considered the main source of PAHs residues ***(Alomirah et al., 2011; Nerín et al., 2016)***. PAHs were significantly produced through meat charcoal grilling process at high thermal contact, and during fat dripping during grilling onto the flames resulting in generating more smoke, or due to pyrolysis of organic matter (Maillard reactions) that form free radicals responsible for PAH formation ***(El-Badry 2010; Singh et al., 2016)***. PAHs hold a significant importance regarding the awareness of their carcinogenicity, mutagenicity, and teratogenicity. The European Food Safety Authority ***(EFSA, 2008)*** estimated the PAH8 (BaA, DBA, BaP, IcP, BbF, BkF, Bghip, and Chr as indicators for the carcinogenicity. Furthermore, the subgroup PAH4 was considered as an adequate marker for the presence of PAHs toxicity in food instead of BaP alone ***(Wretling et al., 2010).*** As well as, PAH8 cannot provide much added value compared to PAH4 (B[a]P), (CHR), (B[a]A), and (B[b]F) ***EC (2011).*** Legislations and regulations for such PAHs were documented by the ***Commission regulation (EU) No 1327/2014),*** as the maximum residue level (MRL) for B[a] P and ΣPAH4 as 2 μg $kg^{-1}$and 12 μg kg^{-1} in the meat. Several individual PAHs like benzo[a]pyrene (B[a]P), are associated with carcinogenic, mutagenic and geno-toxic effects in animal experiments. B[a] P is considered group 1 carcinogenic compounds to human ***(Park et al., 2009).*** Several marinade trails from 2004 to 2020 were carried out and studied their effect in reducing the formation of PAHs on charcoal grilled meat and concluded that the use of the antioxidants, different type of spices and from one hour to two days marination time have a significant effect on reducing PAHs formation ***(Food and Environmental Hygiene, 2004 ; El-Badry, 2010 ; Farhadian et al., 2012 ; USDA, 2013 ; Wong, 2013 ; Abou-Arab et al., 2014 ; HU, 2015 ; Ahmad, 2016 ; Eldaly et al., 2016 ; Jenner, 2017 ; Edikou et al., 2018 ; Anjum et al., 2019; Darwish et al., 2019 and Büyükkurt et al., 2020).*** This study was conducted to assess the effects of various marinade receipts on the grilling time and the levels of polycyclic aromatic hydrocarbons PAH4 and PAH8 formation in charcoal grille whole leg quarters chicken.

Materials and Methods

A total of 13 samples from charcoal whole leg grilled chicken broiler samples after treatment by different four marination processes and grilling (3 samples from each), plus one control sample were collected, after cooling the samples were packed and deep frozen stored at -20^0 C until sample analysis.

Preparation of chicken sample:

Seven fresh slaughtered chicken broilers weighted1.5 kg of each were taken, after complete dressing of the chicken broiler carcasses, whole leg quarters were chosen and marinated for 6 hours in the refrigeration by different marination receipts, then three whole quarters from each process of marination, were grilled over matt glow charcoal with a 15 cm constant distance factor until the core temperature reaches 82̊ C (by using mechanical stainless steel meat thermometer. The time and temperatures were recorded until complete donning of the marinated samples (***J. E Schum GmbH and Co. KG. Ambstein.D-97080 Wurzburg. Germany***

<u>Experimental Trials:</u>

Control sample: The control sample was marinated by 158 gm whit chopped onion, 18 gm pure fine ionized table salt, 5 gm chicken buharrat, 30ml 5% natural cane white vinegar and 2ml fresh lemon juice.

Experiment (1): was marinated by 158 gm white chopped onion, 18 gm pure fine ionized table salt, 5 gm chicken buharrat, 30ml 5% natural cane white vinegar, 2ml fresh lemon juice and 30 ml fresh chopped tomato juice.

Experiment (2): was marinated by 158 gm white chopped onion, 18 gm pure fine ionized table salt, 5 gm chicken buharrat, 30ml 5% natural cane white vinegar ,2ml fresh lemon juice and 5ml of light soy sauce.

Experiment (3): was marinated by 158 gm white chopped onion, 18 gm pure fine ionized table salt, 5 gm chicken buharrat, 30ml 5% natural cane white vinegar ,2ml fresh lemon juice and 2gm of fine ground sage and 2gm rosmarinic.

Experiment (4): was marinated by 158 gm white chopped onion, 18 gm pure fine ionized table salt, 5 gm chicken buharrat, 30ml 5% natural cane white vinegar and 2ml fresh lemon juice ,30 ml fresh chopped tomato juice,5ml of light soy sauce and2gm of fine ground sage and 2gm rosmarinic. The marinated chicken samples were kept for 6 h in the refrigerator at 4-5^{0}C.

The marinated chicken meats were grilled over a charcoal grille with 15cm distance between the chicken meat and the charcoal and kept as a constant factor. A total of 12 samples 25 gm each of 4 charcoal grilled chicken broiler thigh samples (3 samples in each treatment), plus a control sample were collected by using clean instruments and kept in deep freezing storage at -20C until chromatographic analysis.

PAH extraction, analysis and quality assurance:

PAHs were analyzed by the method described before ***(Ikenaka et al., 2008)*** about 10 g of each grilled poultry meat sample was extracted with 25 mL of 1M KOH ethanol solution, and saponified for 10 h at 60°C. The saponified solutions were then shake-extracted 3 times with *n*-hexane and the resulting hexane fractions containing PAHs were run through granular sodium sulphate, evaporated using a rotary evaporator and purified using silica gel column chromatography. The obtained fractions were eluted by 100 mL of acetone/hexane=1/99 (v/v), dried under a gentle nitrogen stream, and re-dissolved into 0.5 mL of methanol for HPL Canalysis. HPLC analysis was performed using a Shimadzu LC20 series (Kyoto, Japan) equipped with a fluorescence detector (RF-10AxL) and a ZORBAX Eclipse PAH (2.1×150 mm, 3.5 lm, Agilent) as a separation column. Identification of PAHs was based on retention time, and quantification was performed by the use of external calibrations which were obtained with PAH solutions at seven concentration levels (0.01, 0.1, 1.0, 10.0, 100.0, 200.0 and 400.0 ng mL−1) for each PAH. Spiking blank sample and heat-treated meat samples with the calibration standards (1.0, 10.0

and 100.0 ng/g) was carried out and all extraction and clean-up steps were repeated. Recovery rates for each PAH congener tested were 85% (acenaphthene), 86% (anthracene), 88% (BaA), 91% (B[*a*]P), 85% B[*e*]P), 82% BbF), 88% (Bo[*ghi*]P) ,85% (B [*k*] F), 95% (Chr), 88% (Dib[*a*,*h*]A) ,103% (fluoranthene) , 87% (fluorene), 84% (naphthalene),91% (phenanthrene), and 84% (pyrene), respectively. The limits for detection (ng/g) of these PAHs were 0.02, 0.03, 0.04, 0.04, 0.02, 0.05, 0.04, 0.05, 0.02, 0.02, 0.04, 0.03, 0.05, 0.02, and 0.05, respectively. The relative standard deviations for replicate analyses (n=3) were below 8%.

Results and Discussion:

The results given in **Table and Fig (1)** revealed that, the minimum, maximum and mean ±SD in control and experiments 1, 2, 3 and 4 for PAH4 (B [a] A; Chr; B[b] F; and B[a] P) were 2.95, 3.90 and 3.45±0.53: 0.19, 0.28 and 0.25±0.04; 0.11, 0.13 and 0.12±0.01; 0.18, 0.41 and 0.29±0.11; 0.04, 0.09 and 0.08±0.00 µg/ kg $^{-1}$respectively. While, **Table (2)** and **Fig (1)** showed also the minimum, maximum and mean ±SD values in control and experiments 1,2,3 and 4 for PAH8 (B [a] A; Chr; B [b] F; B [k] Fe, B [a] P, In (1,2,3c-d)P, Dib(a,h)A and B(g,h,i)P) were 2.85, 4.03 and 3.60 ±0.43 : 0.29, 0.38 and 0.34±0.04 ; 0.18, 0.21 and 0.19±0.01; 0.59, 1.45 and 0.90±0.47 and 0.14, 0.16 and 0.15±0.00 µg/ kg^{-1}respectively. The results obtained for the means of both PAH4 & PAH8 were lower than recorded by ***(Farhadian et al., 2012; Kao et al., 2012; Eldaly et al., 2016; Darwish et al., 2019).*** Nearly, similar results obtained by ***(Ahmad, 2016;; Edikou et al., 2018; Anjum et al., 2019; and Büyükkurt et al., 2020).*** The marination components inhibits the PAH4 and PAH8 formation; this was agreed with ***Jenner (2017) and Haiba et al. (2019).***

The results given in **Table (3)** showed the reduction % of the effect of marinades components used in the experiments 1, 2, 3 and 4 , the reductions were 92.8%, 96.5%, 91.6% and 97.7% ; 90.5%, 94.7%, 75.0% and 95.8%, for both PAH4 and PAH8 respectively. From the results obtained, it showed that the experiment 4 had the most significant reduction (P = 0.001**) effect for both PAH4 and PAH8 in the experimented charcoal grilled chicken, this reduction may attribute to the ripening effect of the different marinades components which led reducing the time and temperature of grilling.

Table (4) showed the correlation coefficient between time and temperature on the formation of PAH4 and PAH8 in marinated charcoal grilled chicken; the correlations were 0.78, 0.82, 0.99 and 1.00; - 0.78, - 0.84, - 0.99 and -1.00. From the statistical analysis data, it showed that a positive correlation indicates that a relationship between time/temperature and the formation of both PAH4 and PAH8 in the experiments 1, 2, 3 and 4 were present and the correlation between time and formation of PAHs in grilled meat are inversed to the temperature in all experiments. The results given in **Table (5)** showed the relationship between time and temperature until the inner core temperature reached 74^{0}C, the time were reduced from 28 minutes in control samples to 15 minutes in the experiments No 4 with 47 % reduction in the grilling time required.

To our knowledge, this is the first study of its kind that provides important information exploring the relationship between cooking time and temperature that are needed until the required donning obtained.

Conclusions: From the results obtained in this study it could be concluded that the long grilling time exposure the more PAHs formation on the chicken meat surface. The fourth type of marination receipt (158 gm white chopped onion, 18 gm pure fine ionized table salt, 5 gm chicken buharat, 30ml 5% natural cane white vinegar and 2ml fresh lemon juice ,30 ml fresh chopped tomato juice,5ml of light soy sauce and2gm of fine ground sage and 2gm rosmarinic) and 6 hours marination time has a power effect on reducing the time needed for reaching the desire donning in the grilled poultry meat (74 ^{0}C), reached 47 % which finally lead to inhibit the amount of PAHs formation in the grilled poultry meat by reducing the exposure time.

Disclosure statement:
No potential conflict of interest was reported by the authors.

Table (1): *Statistical analytical results of PAH4 in the four experimental Samples of charcoals grilled broiler Chicken (µg/kg^{-1})*

Trails	PAH4		
	Minimum	Maximum	Mean ± SD
control	**2.95**	**3.90**	**3.45^a ± 0.53**
Experiment1	**0.19**	**0.28**	**0.25^b ± 0.04**
Experiment 2	**0.11**	**0.13**	**0.12^c ± 0.01**
Experiment 3	**0.18**	**0.41**	**0.29^b ± 0.11**
Experiment 4	**0.04**	**0.09**	**0.08^c ± 0.00**

Table (2): *Statistical analytical results of PAH8 in the four experimental Samples of charcoals grilled whole leg quarter Chicken (µg/ kg $^{-1}$)*

Trails	PAH8		
	Minimum	Maximum	Mean ± SD
control	2.85	4.03	3.60^{a} ± 0.43
Experiment1	0.29	0.38	0.34^{b} ± 0.04
Experiment 2	0.18	0.21	0.19^{b} ± 0.01
Experiment 3	0.59	1.45	0.90^{b} ± 0.47
Experiment 4	0.14	0.16	0.15^{b} ± 0.00

One-way ANOVA (F-test) was conducted for analyzing the above data, followed
by Duncan's and Tukey's tests as the post hoc tests for mean separation. Within the same row, means with different superscripts are significantly differ ($P < 0.01$).

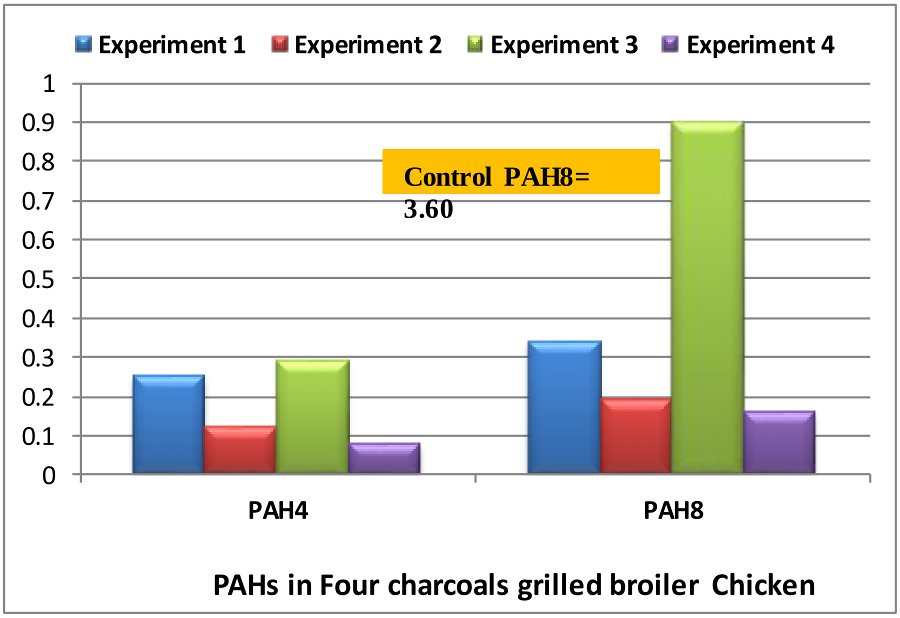

Figure (1): *Means values of PAH4 and PAH8 of charcoals grilled whole leg chicken quarters.*

Table (3): *Reduction % of the PAHs in examined marinated charcoal grilled chicken samples.*

Charcoal grilled Chicken		Mean ± SD	Reduction %
PAH4	Control	$3.45^a \pm 0.53$	-
	Experiment 1	$0.25^b \pm 0.04$	92.8 %
	Experiment 2	$0.12^b \pm 0.01$	96.5 %
	Experiment 3	$0.29^b \pm 0.11$	91.6 %
	Experiment 4	$0.08^b \pm 0.00$	97.7 %
P = 0.001**			
PAH8	Control	$3.60^a \pm 0.43$	-
	Experiment 1	$0.34^b \pm 0.04$	90.5 %
	Experiment 2	$0.19^b \pm 0.01$	94.7 %
	Experiment 3	$0.90^b \pm 0.47$	75.0 %
	Experiment 4	$0.15^b \pm 0.00$	95.8 %
		P = 0.001**	

Table (4): *The relationship between time and temperature min/74°*

Control	Experiment 1	Experiment 2	Experiment 3	Experiment 4
Tim/Min	Tim/Min	Tim/Min	Tim/Min	Tim/Min
28	19	18	17	15

Table (5): *Correlation coefficients for association between Times, Temperature and PAHs in marinated charcoal grilled chicken*

Trails	PAH4 Vs. Time	PAH4 Vs. Temp	PAH8 Vs. Time	PAH8 Vs. Temp
Experiment 1	0.78	- 0.78	0.78	- 0.78
Experiment2	0.82	- 0.84	0.76	- 0.79
Experiment3	0.99	- 0.99	0.99	- 0.99
Experiment 4	1.00	- 1.00	1.00	-1.00

Positive correlations indicate positive relationship between any two variables

Negative correlations indicate inverse relationship between any two variables

Correlation coefficient < 0.5 indicates weak relationship

Correlation coefficient ~ 0.50 indicates moderate relationship

Correlation coefficient ≥ 0.75 indicates strong relationship

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الملخص العربي

التأثير التثبيطي للمتبلات على تواجد المركبات الهيدروكربونيه العطريه متعددة الحلقات في أفخاذ الدجاج اللاحم المشوي على الفحم

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أجريت هذه الدراسة لتقييم التأثيرات المثبطة للتتبيلات المختلفة على وقت الشواء ودرجه الحراره علي مستويات تكوين المركبات الهيدروكربونيه العطريه متعددة الحلقات لكل من PAH4 و PAH8 في افخاذ الدجاج المشويةعلى الفحم لما لهما من تأثير سرطاني محتمل ومؤكد في الإنسان. و كانت قيم متوسطاتها في نتائج التجارب والعينة الضابطة و 1 و 2 و 3 و 4 لـPAH4 (بنزو [أ] أنثراسين؛ كريزين؛ بنزو [ب] فلورانثين؛ بنزو [ك] فلورانثينوبنزو[أ]بيرين) هي 3.45 ± 0.53 ، 0.25 ± 0.04 ، 0.12 ± 0.01 ، 0.29 ± 0.11 و0.08 ± 0.00 ميكروغرام / كغ-1 على التوالي. بينمـا كانت بالنسبة لـ PAH8 (بنزو [أ] أنثراسين؛كريزين؛بنزو [ب] فلورانثين؛بنزو [ك] فلورانثين؛بنزو[أ]بيرين،إنديدنو (1،2،3 ج-د) بيرين،دايبنزو (أ،ح) أنثراسينو البنزو(ج, ه,ي) بيريلين) تكون 3.60 ± 0.43 ، 0.34 ± 0.04، 0.19 ±0.01 ، 0.90±0.47 و 0.15± 0.00 ميكروغرام / كغ-1على التوالي.

أظهرت نتائج تأثير التتبيلات المختلفة في التجارب 1 و 2 و 3 و 4 تقليل نسب تكون ال PAH4في العينات المتبله ب 92.8٪ و 96.5٪ و 91.6٪ و 97.7٪ ,علي التوالي. وبالنسبه ل PAH8 كانت نسبه الاختزال هي 90.5٪ ، 94.7٪ ، 75.0٪ و 95.8٪ على التوالي. كما أظهرت النتائج أن التجربة 4 كان لها أكبرنسبة اختزال (** P = 0.001) لكل من PAH4و PAH8 في الدجاج المشوي على الفحم وذلك نتيجة لتأثير التتبيلات المختلفة علي اتمام عملية النضج للحوم الدواجن مما ادي الي تقليل تعرضها لفتره طويلة علي الفحم اثناء عميلة الشواء وان هناك علاقة وثيقة بين زمن ومدة تعرض لحوم الدجاج للحرارة وتكوين المركبات الهيدروكربونيه العطريه في لحوم الدجاج المشوي علي الفحم.