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Food safety issues in relation to Polycyclic Aromatic Hydrocarbon (PAHS) content with special reference to control trial

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ARTICLE INFO	ABSTRACT		
Keywords	This study aimed to examine seventy-five different grilled chicken meats, represented by		
Food safety	charcoal grilled chicken meat, grilled shawarma, and shish tawook on a heated grill (twenty- five from every type). The meat samples were collected from random restaurants located in Al-		
HPLC	Kalyobiya governorates, Egypt, to examine some polycyclic aromatic hydrocarbon (PAHs) by High-Performance Liquid Chromatography (HPLC) technique, and evaluation of their contents		
Hydrocarbon	against European Commission regulations standards limit "2 µg /Kg for Bap and 12 µg /Kg for PAH4". Regarding the obtained findings, charcoal grilled samples showed the peak levels of		
Meat products	PAHs and PAH4 that were recorded as 20.71 \pm 2.04 μg /Kg and 10.32 \pm 0.95 μg /Kg, respectively, followed by shawarma "17.09 \pm 1.26 μg /Kg for PAHs and 8.26 \pm 0.69 μg /Kg		
Ready-to-Eat	for PAH4" and sheish tawook samples " $11.23 \pm 1.26 \ \mu\text{g}$ /Kg for PAHs and $5.53 \pm 0.44 \ \mu\text{g}$ /Kg for PAH4". The application of thyme extract as marination in chicken meat preparation before grilling revealed significant reductions in total PAH4 levels by about 23.1%,46.8%, and 65.6%		
Received 20/12/2024	respectively in samples treated with thyme oil with concentrations of 0.5%, 1%, and 1.5%		
Accepted 06/01/2025	respectively, revealing it a promising treatment for safer grilled chicken meat products.		
Available On-Line	Ultimately, the ongoing surveillance revealed that some grilled commercial ready-to-eat-		
01/04/2025	chicken meat products in Egypt are safe for human consumption in terms of PAH levels. Moreover, adding thyme as chicken meat marinade is a promising safe, natural way to diminish the level of PAHs in grilled chicken meat products.		

1. INTRODUCTION

Meat products refer to meat that has been undergo different methods of conservation like curing, smoking, dehydration or other manners to enhance flavor or improve preservation. Meat products play a part in the human diet, with their consumption experiencing a notable rise worldwide in recent years. These foods are an excellent source of energy and provide essential nutrients, including vital amino acids, high-quality proteins, minerals such as iron, zinc, selenium, and manganese, as well as B-complex vitamins, particularly vitamin B12. They are also crucial for sustaining the needs of modern society as income levels rise, particularly in developing countries, so there is a corresponding increase in meat consumption (Molina et al., 2024). Even with these values, the perception of processed meat products among consumers has turned negative; as the recent studies have revealed significant links between consumption of processed meats and various health risks, including pancreatic and colorectal cancer (IARC, 2015).

Processed meat increases the risk of cancer due to the production of various carcinogenic chemicals during processing and cooking. One significant group of these chemicals is polycyclic aromatic hydrocarbons (PAHs), which are primarily formed when meats are cooked at high temperatures (Bulanda and Janoszka, 2022). Polycyclic aromatic hydrocarbons (PAHs) consist of compounds over one-hundred fused- aromatic ring that are produced from the incomplete incineration of organic materials, the thermal decomposition of commonly utilized energy sources, industrial incineration, and emissions from tobacco smoke and vehicle exhaust (Zhang et al., 2022). Heat treatments of meat, such as grilling, barbecuing, pan-frying, and smoking, can lead to the release of increased levels of PAHs, significantly contributing to consumer exposure to these compounds. Factors such as cooking method, temperature, duration, fat content, and oil type all affect the formation of PAHs. Grilling and cooking over charcoal produce particularly high concentrations of PAHs due to the pyrolysis of organic matter at elevated temperatures (Sampaio et al., 2021). The exact mechanisms behind the formation of PAHs in food remain uncertain. Nonetheless, it is widely acknowledged that their production results from complicated reactions involving free radical-mediated pyrolysis and thermal synthesis of organic materials, including fats, proteins, and carbohydrates (Yu et al., 2023). Several factors have a clear role in the rate of PAHs in food as the type of food, preparing method, period during cooking and temperature, and food additives (Siddique et al., 2021). So, the IARC association, part of WHO, announced that it has classified processed meat as 'teratogenic to humans' (Group I). Additionally, it is halted that each fifty-gram section of processed meat consumed per day upturns the risk of colorectal cancer by eighteen percent (IARC, 2015).

PAHs are synthesized from precursors and their synthesis includes free radical reactions, but antioxidants neutralize

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free radicals formed during cleavage of hydrocarbon and cyclization of aromatic compounds, thereby counteracting the formation of PAHs (Onopiuk et al., 2022). Consequently, the insertion of antioxidants to meat products is considered a proficient way to minimize the intensities of PAHs in cooked meat products. Customer choice is inclined toward natural antioxidants which are also present in plenty of plant sources such as tea, spice, vegetables, fruits and their waste. So recently, the application of plant extracts as natural and safe antioxidants is also effective in preventing the formation of PAHs in the processing of meat products (Shen et al., 2022).

Therefore, there is significant interest and a pressing need for not only the production and processing of high-quality meat but also for ensuring its safety and suitability for human consumption in the long run. Therefore, this study pointed out to detect the prevalence of some PAHs concentration in some commercial RTE chicken meat products represented by charcoal grilled chicken meat, shawarma and sheish tawook samples collected from random restaurants located in Benha city, Kalyobiya governorate, Egypt by HPLC technique. Moreover, experimental trial was accomplished to explore the inhibitory effect of thyme oil on the potential development of PAHs in grilled chicken meat products.

2. MATERIAL AND METHODS

All procedures used in this investigation was approved by Scientific Research Ethics Committee, Faculty of Medicine, Benha University with Ethical Approval Number (re 10-8-2024).

2.1. Sampling

Seventy-five random samples of chicken meat products represented by charcoal grilled chicken meat, shawarma, and sheish tawook (25 of each) were gathered from diverse restaurants in Benha city, Kalyobiya governorate, Egypt. The collected samples were analyzed for determination of their Polycyclic Aromatic Hydrocarbons (PAHs). The difference of such serious pollutants in the examined samples with the safe permissible limit recorded by European Commission regulations (EU) was applied to evaluate their fitness for human consumption. Additionally, certain trials using thyme oil to control such residues were performed.

2.2. Detection of Polycyclic Aromatic Hydrocarbons (PAHS)

The sample preparation process was outlined in line with Stumpe et al. (2008). To 25 g of the sample, 12 g of potassium hydroxide, 100 ml of ethanol, 25 µl of 10 ng/µl conc. Internal standard benzo[a]pyrene-d12 solution and 125 µl of 1ng/µl conc. PAH were added and mixed well. The mixture was then subjected to alkaline treatment with potassium hydroxide and ethanol by heating for 2 hours at 40°C under reflux and filtered. The cyclohexane extract was mixed with 50 ml of a N,N-dimethylformamide/water solution (in a9:1 ratio). The resulting layer of the N,Ndimethylformamide/water solution was transferred to a 250 ml separating funnel, where 50 ml of a 1% NaCl solution was added. PAHs were then extracted using 75 ml of cyclohexane. The cyclohexane phase was dried over anhydrous sodium sulfate and concentrated using a rotary evaporator under reduced pressure. The extract was then

applied to a silica solid-phase extraction (SPE) column preconditioned with 5 ml of cyclohexane. The flask was bathed with an additional 3 ml of cyclohexane, and the PAHs were eluted with 6ml of cyclohexane.

Determination of PAHs was accomplished using an Agilent 1200 HPLC system (Germany), with detection limits of 2.8 ng/g for PAHs. The recovery results of PAHs from the various meat products under study were assessed based on the method employed by Chantara and Sangchan (2009).

2.3. Experimental part using of thyme oil for control PAHs

Accurately, 20 samples of chicken meat were represented by untreated 5 samples (control), 15 samples were supplemented with thyme oil at concentrations of 0.5%,1% and 1.5% (5 of each). All treated samples were sprayed with the applied thyme oils (0.5%, 1%, and 1.5%) and left for 15 minutes. Accordingly, all the tested samples either control or treated were cooked by ordinary charcoal grilling. Therefore, all grilled chicken meat samples were scanned for determination of their contents of BaP, BaA, BpF and CHR Polycyclic Aromatic Hydrocarbons (PAH4) most carcinogenic PAHs. The reduction of PAHs was estimated to establish the consequence of such treatment on the formation of such dangerous residues. Reduction (%) = $\left(\frac{(R1-R2)}{R1}\right) x$ 100, where R1 and R2 indicate the PAH level of control and thyme-treated samples, respectively.

2.4. Statistical analysis

The obtained data was analyzed statistically by one-way analysis of variance (ANOVA) employing the Statistical Package for the Social Sciences (SPSS) program for Windows version 16 (2007) (SPSS Inc. Chicago, IL and USA) Later, Duncan's post hoc test was applied for comparison of the means, where the level of significance set at $P \le 0.05$ was deemed statistically significant.

3. RESULTS

Figures (1-3) illustrate that benzo[a]anthracene (BaA) exhibited the highest concentration among the analyzed polycyclic aromatic hydrocarbons (PAHs) in the chicken meat products examined. The mean concentrations of BaA in the grilled, shawarma, and sheish tawook samples were 4.91, 4.13, and 2.67 μ g/kg, respectively. Furthermore, grilled chicken samples showed higher PAH levels compared to the other samples, with statistically significant differences (P \leq 0.05) observed among the products concerning PAH4, PAH8, and total PAH values, indicating a significant fitness parameter (Fig. 4)

PAH4 values (BaA+BaP+BbF+CHR) were summed and calculated for estimation of the examined products safety for human consumption. Recorded mean values in Fig. (4) indicated that charcoal grilled samples recorded the highest PAH4 concentration (10.32 μ g\kg), followed by shawarma (8.26 μ g\kg) and sheish tawook came the least (5.53 μ g\kg); therefore, and in reference to the European Commission regulations (EU), it found that 44, 40 and 16% of the examined samples recorded higher sum value of PAH4 than the permissible limit considering them unfit for human consumption (Fig. 5).

According to the recorded findings of the expérimental trial for diminishing of PAHs arise from charcoal chicken meat grilling (Table 1), their is a significant reductions in PAHs levels in thyme treated groups were recorded in concentration dependant manner, where higher thyme concentration (1.5%) made higher reduction in PAHs level.

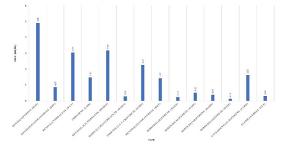


Fig. (1). The mean values of PAHs levels in the examined charcoal grilled chicken meat

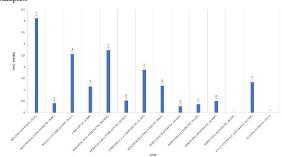


Fig. (2). The mean values of PAHs levels in the examined chicken shawarma samples

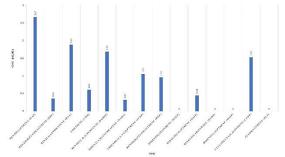


Fig. (3). The mean values of PAHs levels in the examined chicken shiesh-tawook samples.

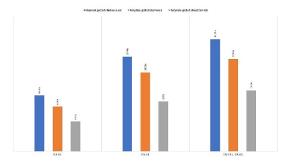


Fig. (4). The mean values of PAH4, PAH8, and total PAHs levels in the examined chicken meat samples. PAH4: BaP, BaA, Bbf & CHR. PAH8: BaP, BaA, Bbf, CHR, BkF, DahA, BghiP & IcdP. a.b. c.d Different superscript letters indicating significant difference when $P \leq 0.05$.

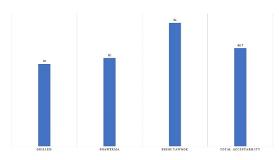


Figure (5): Prevalence (%) of accepted samples of chicken meat products based on their PAH4 contents (n=25). * Maximum Residual Limit of PAH4 stipulated by Commission Regulation "EU" (2023) (12 μ g/Kg).

Table (1) Reduction (%) of PAHS concentrations in the thyme-treated charcoal grilled chicken meet samples (n-5)

	T 0.5%	T1.0%	T 1.5%
Benzo[a]Pyrene (BaP)	19.8	44.2	63.9
Benz[a]anthracene (BaA)	28.1	49.1	68.4
Benzo(b)fluoranthene (Bbf)	16.7	37.5	58.3
Chrysene (CHR)	31.6	52.6	73.7
Sum PAH4	23.1	46.8	65.6

4.DISCUSSION

Grilled meat products are created from both fat and muscle of wholesale-cuts, as well as non-muscle parts like liver. The grilling process allows the meat to absorb volatile compounds produced by the thermal decomposition of charcoal, which in turn generates phenolic compounds that play a significant role in enhancing the flavor and aroma of smoked meats (Lonergan et al., 2019). The presence of polycyclic aromatic hydrocarbons (PAHs) in processed meat and meat products is a global concern as they are known to be carcinogenic, mutagenic, teratogenic, and genotoxic to living beings. Polycyclic aromatic hydrocarbons (PAHs) are environmental pollutants that derive from the pyrolysis or incomplete combustion of organic matter (IARC, 2015). A 10-year study concluded that extreme utilization of processed meat was related closely to an overall increased mortality risk, which was attributed to the carcinogenic risk of cyclic amines, polycyclic aromatic hydrocarbons and saturated fat. In experimental animals, PAH benzo[a]pyrene (BaP) showed various toxicological and carcinogenic effects including haemato-toxicity, reproductive, developmental toxicity, and immunotoxicity (Sivasubramanian et al., 2023).

The current study focused on PAH4, which encompasses the total content of benzo[a]pyrene (BaP), benzo[a]anthracene (BaA), chrysene (CHR), and benzo[b]fluoranthene (BbF). Additionally, the concentrations of 14 other PAHs were analyzed, in accordance with the European Commission Regulation (EU) (2023), which establishes maximum permissible levels for PAH4, BaA, and BaP in meat products at 12 µg/kg, 2 µg/kg, and 5 µg/kg, respectively. Furthermore, a specific limit for BaA is set at 2 µg/kg. Based on their degree of toxicity, Benzo[a]pyrene(B[a]P) is recognized as the most probable human carcinogen among different fractions of PAHs by the European Commission Regulation (EU) (2023). The results indicate that while benzo[a]pyrene (BaP) exhibited the highest mean values among the detected polycyclic aromatic hydrocarbons (PAHs) in the examined processed chicken meat samples, these levels remained within the permissible limits established by the European Commission Regulation (2023). This finding is consistent with the results reported by Khalili et al. (2023), who also found that BaP levels in all examined samples were within acceptable limits. In contrast, Sahin et al. (2020) reported lower BaP levels in grilled chicken meat samples compared to other examined samples, while Abdel-Latif et al. (2022) did not detect BaP in any of the analyzed chicken samples. In a comparison of the mean PAH values among the examined products, grilled chicken samples exhibited the highest concentrations of both total PAHs and PAH4, indicating a greater potential hazard to consumers with prolonged consumption. This was followed by shawarma and sheish tawook, respectively. The elevated levels in grilled chicken may be attributed to factors such as direct exposure to volatile products from charcoal combustion, duration of exposure, and processing temperature, as well as the quality and completeness of the combustion and smoking processes (Sampaio et al., 2021). Moreover, it came in line with the recorded results of Ali et al. (2022) who reported

that benzo[a]pyrene, along with the sum of benzo[a]pyrene, benzo[a]anthracene, benzo[b]fluoranthene, and chrysene (collectively referred to as PAH4), as well as the genotoxic PAHs (PAH8) and total PAHs (SPAHs), exhibited the highest average concentrations in charcoal-grilled chicken samples, measuring 1.38, 3.09, 3.09, and 36 µg/kg, respectively. In contrast, the lowest mean values were observed in chicken samples grilled using electric machines, which recorded levels of 0 µg/kg for benzo[a]pyrene, 0.44 µg/kg for benzo[a]anthracene, 0.55 µg/kg for benzo[b]fluoranthene, and 26.36 µg/kg for total PAHs. While, lower levels were recorded by Hussein et al. (2023) who recorded that the PAH4 and total PAHs of the examined samples were 66.37 and 35.56 µg/kg, respectively.

Furthermore, estimation of the compatibility of the examined samples for human consumption in relation to the PAH4 concentrations, where sheish tawook samples showed the highest compatibility (84.0%) with lower PAH4 value (5.53µg\kg), followed by shawarma and charcoal grilled samples with compatibility percent in 60.0 and 56%, respectively. Also, benzo[a]pyrene exceeded the permissible limits in 40% of charcoal-grilled chicken samples. In comparison to previously reported data, the concentrations of PAHs and PAH4 observed in this study can be contrasted with findings from Barakat (2021), who investigated the occurrence of various carcinogenic compounds, including PAHs, in processed meat products. Barakat found of benzo[a]pyrene concentrations (BaP). benzo[a]anthracene (BaA), chrysene (CHR), and PAH4 at levels of 1.74, 1.83, 0.83, and 4.4 µg/kg, respectively. Notably, benzo[b]fluoranthene (BbF) was not detected in the grilled meat samples analyzed from Assiut City, Egypt. Additionally, Hamidi et al. (2022) reported a mean total PAH value of 7.1 µg/kg in charcoal-grilled chicken. The variation between different authors may be attributed to variation in collection localities, type of smoking and processing, types of the examined samples, and time of exposure.

PAHs are generated in processed meat through different thermo-processing techniques, such as smoking, grilling, barbecuing, roasting, and frying, which involve abnormal high-temperature treatments and extruded fuels. These carbonaceous compounds are highly stable and toxic, and their generation is enhanced by faulty thermal processing techniques, contaminated raw materials, and environmental pollution. Furthermore, the association between dietary PAHs exposures and their role as carcinogen in human beings has been reported clinically. Therefore, it is necessary to focus on prevention and control of PAHs formation in processed meat products through various strategies to avert public health concerns and safety issues. Accordingly, several approaches have been used to reduce the risk of PAHs formation such as marination by natural plant components to eliminate or reduce the harmful effects of PAHs in the food system (Das et al. 2023).

The influence of chicken meat marination with different concentrations of thyme oil was observed against PAHS concentrations in the examined samples of experimentally grilled chicken meat, significant reductions in BaP, BaA, BbF, CHR, and PAH4 were recorded in the thyme-treated groups compared to the control untreated group. Reductions were strongly correlated to the concentration of thyme in the prepared marinade, where higher thyme concentration made a more diminishing effect on the detected PAHs in the examined samples.

Many previous studies reported the promising diminishing effect of different plant extracts addition in meat marinade pre-grilling, such as Jeong et al. (2018) who studied the

beneficial effect of marination of different types of meats with thyme extract alone or in combination and recorded a significant improvement in the sensory and chemical quality of the treated samples with significant inhibition on PAHs levels. In addition, Onopiuk et al. (2022) investigated the effect of the inclusion of natural plant extracts such as bay leaf, black pepper, turmeric, jalapeno pepper, and tamarind paste in marinades and charcoal grilling (12 min) of pork between 280 and 300 °C; and found that Jalapeno pepper marinade had statistically significant reducing effect (95% reduction) on total sum of PAHs levels. In addition, Hussein et al. (2023) investigated the diminishing effect of thyme oil (0.5, 1.0, and 1.5%) on the PAHs concentrations in treated beef samples, where PAHs were reduced by 39, 58 and 74%, respectively proved that the diminishing effect of thyme oil is dose-dependent. The authors concluded that biologically active substance herbal extracts have acted as scavengers of produced free radicals, which prevented the cyclisation and oxidation reactions thereby enhancing the safety and shelf life of grilled meat products.

The findings of this study underscore the promising effect of using plant extracts such as thyme oil in decreasing the hazardous effect of PAHs formed in chicken meat during processing. keeping in mind that the strategies used should not drastically alter/affect the sensory characteristics of meat products as mentioned by Singh et al. (2023).

5. CONCLUSIONS

In conclusion, although the current examined samples appeared to be within the safe limit of BaP, about 44%,40%, and 16% respectively of the examined grilled chicken meat, Shawarma, and Sheish tawook respectively were unfit for human consumption based on their PAH4 content. Charcoal grilled chicken meat samples had higher PAHs contents with mean value $20.71 \pm 2.04 \,\mu$ g/kg than the other examined meat products that recorded with mean 17.09 $\pm 1.26 \,\mu$ g/kg for chicken sheish tawook samples. Application of thyme oil as chicken meat marinade for 15 minutes showed significant reductions in PAHs formation after grilling, that indicates its promising inhibitory effect on PAHs formation in grilled chicken meat products.

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