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TRIALS TO ESTIMATE AND CONTROL THE RESIDUAL LEVELS OF HETEROCYCLIC AROMATIC AMINES IN MEAT PRODUCTS

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ABSTRACT

One hundred and twenty samples of meat products represented by grilled kofta, grilled meat, charcoal grilled kofta, and charcoal grilled meat (30 pieces each) were randomly collected from different markets in Kalyobia Governorate, Egypt. Each sample was individually packed in a plastic bag and taken directly to the laboratory. Collected samples were analyzed for heterocyclic aromatic hydrocarbons (HAAs). This study aimed to reduce the amount of carbonization and fat pyrolysis by reducing the amount of meat droplets using antioxidants such as butylhydroxytoluene (0.02%) and carvacrol (1%). The observed findings showed that the average levels of HAAs (µg/kg) in charcoal-grilled meat samples, left untreated (n = 30), were as follows: BhIP, MelQx, 4,8-DiMelQx and Norharman were Norharman while IQ was beyond the undetected limit (UDL). Meanwhile, the treated samples with (BHT 0.0% and carvacrol 1%) showed that individuals; BhIP, MelQx, 4,8-DiMelQx, and Norharman were 3.8±0.3, 0.5±0.1, 1.2±0.1 and 1.8±0.1 with reduction percentage 41.5%, 28.6%, 36.8% and 47.1%, respectively for BHT concentration 0.02%. While for carvacrol concentration 1% individuals; BhIP, MelQx, 4,8-DiMelQx, and Norharman were 3.1±0.2, 0.4±0.2, 1.0±0.1 and 1.5±0.1 with reduction percentages 52.3%, 42.9%, 47.4%, and 55.9%, respectively. In conclusion, this study recommends the treatment of charcoal grilled meat with antioxidants, such as BHT 0.0% and carvacrol 1%, to reduce HAAS concentrations in the examined samples.

Keywords: Heterocyclic Aromatic Amines (HAAs), charcoal grilled meat, charcoal grilled kofta, BHT, Carvacrol

INTRODUCTION

As sugars, creatine, and amino acids react at temperatures higher than 150 °C, heterocyclic amines (HCAs) are produced. When high-temperature cooking methods like frying, grilling, or cooking directly over an open flame are applied to protein-rich foods, particularly those that contain animal muscle tissue like meat and fish, HCAs are produced. Temperature, cooking time, and type of meat product all have a major impact on the amount of HCA present. In smaller amounts, these HCAs can also be found in baked and fried foods made with grains, as

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well as cooked red meat, fish, and chicken (Adeyeye, 2020).

Researchers and consumers alike are very concerned about the presence and potential danger of HCA compounds in processed meat products. A class of carcinogenic substances known as heterocyclic amines (HCAs) was discovered in foods high in protein in the 1970s (Rahman *et al.,* 2014). Five of them are classified as moderate human carcinogens by the IARC (1993); These include 2-amino-3-methylimidazo[4,5-f]quinoline (IQ), 2-amino-3,4-dimethylimidazo[4,5-f]quinolone (MeIQ), 2-amino-3,8-Dimethylimidaze [4,5-f] quinoxaline (MeIQx), 2-amino-3,4,8-trimethylimidaze [4,5-f] quinoxaline (4,8-DiMeIQx), and 2-amino-1-methyl - 6-Phenylimidase[4,5-b]pyridine (PhIP).

HAAs were initially identified in beef in 1977 because of common home cooking procedures by Professor Sugimura and his associates (Norat *et al.,* 2002). This class of chemicals includes twenty distinct compounds (Sugimura, 1997).

The amount of HAA formed in cooked meat depends on several factors, including the type of meat, cooking temperature, cooking time, and degree of browning (Skog *et al.,* 1998).

Another factor that affects the production of HAA in meat is the cooking method. Studies have shown high levels of HAA in beef samples fried, roasted, broiled, fried, or grilled (Cross and Sinha, 2004).

According to studies, eating meat containing heavy amino acids (HAA) can cause cancer in both humans and animals. For example, eating meat containing HAAs can lead to skin, breast, liver, colon, prostate, lung, and gastrointestinal cancers (Sugimura *et al.,* 2004).

Managing the production of HAAs requires using different strategies. These cancer-causing chemicals can be reduced by pretreating meet with microwave radiation, avoiding direct flame contact with meet surfaces, and turning meet frequently during cooking. Furthermore, a variety of herbs, spices, fruit, and root extracts can help lower the production of HAAs (Hala, 2011).

In this study, we use antioxidants such as butylhydroxytoluene and carvacrol to regulate HAA levels and control carcinogenic effects in charcoal grilled meat.

MATERIAL AND METHODS

In compliance with ethical guidelines (NO BUFVTM 40-06-23), this study was conducted at the Center of Experimental Animal Research, Faculty of Veterinary Medicine, Benha University, Egypt.

collection of samples

One hundred and twenty randomly selected samples of meat products, comprising 30 pieces of each of grilled kofta, grilled meat, charcoal grilled kofta, and charcoal meat, were gathered from various markets within the Kalyobia Governorate of Egypt. Every sample was carefully wrapped in a plastic bag before being delivered directly to the laboratory. Heterocyclic aromatic hydro-carbons (HAAs) were detected in the samples.

To find out the suitability of the examined meat products for human consumption, the residue limits established by international laws were compared with the levels of harmful residues found in the examined meat products. In addition, some experiments have been conducted using antioxidants and natural oils to control those residues that lead to excessive meat products.

Determination of Heterocyclic Aromatic Amines (HAAs):

Chemicals

- PhIP(CAS no:105650-23-5), - IQx (CAS no: 108354-47-8),
- MeIQx (CASno: 77500-04-0), - 4,8- DiMeIQx (CAS no: 95896-78-9)
- IQ (CAS no: 76180-96-6), **(Milex, Massachusetts, USA)**

The traditional stock solutions were prepared using Öz *et al.* (2010)'s methodology. Solids were extracted from ultra-filtered waters using an Oasis MCX cartridge (3 cm3/60 mg, 30 µm). The remaining chemicals were all analytical or HPLC grade. The source of the water was a Milli-Q water filtration system. Each solution underwent filtration using a 0.45-µm filter. (Milex, Massachusetts, USA).

Extracting HAAs

The procedures recommended by Messner and Murkovic (2004) for the extraction of HAAs from the materials under study were followed, albeit with a few minor modifications. Meat and meat products contain complex matrices containing HAAs only at ppb concentrations, which affects sample preparation. It is challenging to accurately and sensitively quantify these substances. The separation methods based on HAA adsorption are incredibly selective.

One gram of the sample was to be dissolved in 12 ml of 1 M NaOH according to the protocol. A magnetic stirrer was used to homogenize the suspension for an hour at 500 rpm while it was at room temperature. The alkaline solution was mixed with 13 g of diatomaceous earth (Extrelut NT packaging material, Merck, Darmstadt, Germany), and then the mixture was poured into empty Extrelut columns.

Following ethyl acetate extractions, the eluate was filtered using equipped Oasis MCX cartridges. We washed the cartridge with 2 ml each of 0.1 M HCl and MeOH. The analytes were eluted using two milliliters of 25% ammonia (19/1, v/v) concentrated in MeOH. The eluted mixtures were dried at 50 °C before the final extracts were dissolved in 100 µL MeOH for measurement.

Detection and Measurement of HAAs

Specifically, an Agilent 1200 HPLC system (Germany) comprising a quaternary pump G1311A, a vacuum degasser G1322A, an automatic injector G1329A with sample tray G1330A, and other components was used to identify and quantify HAAs.

An analytical column in reverse phase, Acclaim TM 120 C18 3 µm (4.6 x 150 mm) from Tosoh Bioscience GmbH in Stuttgart, Germany, was used for the separation. It was carried out at 30°C using acetonitrile, water, methanol, and acetic acid (8/14/76/2, v/v/v/v) as the mobile phase and acetonitrile as solvent B at a flow rate of 0.7 ml/min. The pH of the mixture was adjusted with 25% ammonium hydroxide. The gradient method involved starting with no B for the first 12 minutes, increasing from 0% to 30% B for the next 20 minutes, and staying at 30% B for the final 25 minutes. Using a 10µl injection volume and a wavelength of 262 nm, DAD was used to detect HCAs.

Table 1: Displays each HAA's LOD and LOQ values.

HAAs	LOD (µg/Kg)	LOQ (µg/Kg)
BhIP	0.025	0.085
MelQx	0.024	0.081
4,8-DiMelQx	0.008	0.025
Norharman	0.015	0.040
IQ	0.009	0.029

Experimental part

Using of antioxidants (BHT and carvacrol) for control of HAAs

Nine samples of meat fillets were represented by untreated 3 samples (control), 3 samples treated with 0.02% butylated hydroxyl Toluene, and 3 samples treated with 1% carvacrol (BHT and Carvacrol is obtained from NieLife Co., PO Box 49638, Sheikh Zayed, Egypt). The preceding meat samples, both the treated and control, were wrapped individually in plastic bags and labeled. To ascertain each group's HAAs, an examination was conducted.

Analytical Statistics

The data gathered was assessed through ANOVA with Duncan using SPSS® version 16.0 as outlined by Feldman *et al.* (2003).

RESULTS

Heterocyclic aromatic amines are substances produced when meat is cooked to high temperatures. These compounds are formed in large amounts depending on the type of meat, cooking temperature, cooking time, and cooking method. The structural elements of heterocyclic amines are nitrogen-containing groups and heterocyclic rings. No doubt using heterocyclic amines raises the risk of developing cancers of the skin, breast, colon, pancreas, gastrointestinal tract, lung, liver, and prostate. To counteract these mutagenic chemicals, add extracts from different fruits and vegetables. Antioxidants and a range of spices can also be used to lower these heterocyclic amines.

Based on the outcomes shown in Table 1, the mean concentration levels of potentially carcinogenic HAAs levels (µg/kg) in the evaluated samples of roasted kofta (n=30) individuals; BhIP was 0.71 ± 0.04, while 4,8-DiMelQx and IQ were UDL.

According to Table 1 outcomes, the average levels of HAAs concentration (µg/kg) within the investigated samples of roasted meat (n=30) individuals; BhIP, MelQx, and Norharman was 0.54 ± 0.02µg/kg, while 4, 8-DiMelQx and IQ were UDL.

As can be seen in Table 1, the samples of charcoal-grilled kofta (n=30) that were analyzed had estimated HAAs levels (µg/kg) of 15.39 and 9.22 µg/kg for BhIP and Norharman, respectively. Table 2's findings showed that the average levels of HAAs (µg/kg) in the samples of charcoal-grilled meat (n = 30) were as follows: IQ was UDL, while BhIP, MelQx, 4,8-DiMelQx, and Norharman were all Norharman.

Additionally, the findings of the present investigation presented in Table 2 demonstrated the influence of antioxidants (BHT 0.02% and carvacrol 1%) on HAAs (µg/kg) in the samples that were analyzed of charcoal grilled meat fillets (n=5) individuals; BhIP, MelQx, 4,8-DiMelQx and Norharman were 3.8±0.3, 0.5±0.1, 1.2±0.1 and 1.8±0.1 with reduction percentage 41.5%, 28.6%, 36.8% and 47.1%, respectively for BHT concentration 0.02%.

While for carvacrol concentration of 1% individuals; BhIP, MelQx, 4,8-DiMelQx, and Norharman were 3.1±0.2, 0.4±0.2, 1.0±0.1 and 1.5±0.1 with reduction percentage 52.3%, 42.9%, 47.4%, and 55.9%, respectively.

Table 2: Examination of HAA levels (µg/kg) in analyzed samples (n=30).

HAAs	Roasted kofta (Mean ± S.E*)	Roasted meat (Mean ± S.E*)	Charcoal grilled kofta (Mean ± S.E*)	Charcoal grilled meat (Mean ± S.E*)
BhIP	0.71 ± 0.04	0.54 ± 0.02	15.39 ± 1.05	Norharman
MelQx	0.46 ± 0.01	0.25 ± 0.01	2.08 ± 0.16	Norharman
4,8-DiMelQx	**UDL**	**UDL**	3.73 ± 0.31	Norharman
Norharman	0.19 ± 0.01	0.13 ± 0.01	9.22 ± 0.67	Norharman
IQ	**UDL**	**UDL**	0.35 ± 0.02	**UDL**

UDL: Undetected limit.

Values were expressed as Mean ± Standard error

Table 3: Effect of two antioxidants (BHT and carvacrol) on levels of HAAs (µg/kg) in five samples of charcoal grilled meat fillets studied.

Treatment / HAAs	Control	0.02 % BHT		1% carvacrol	
	Average level	Mean	R %	Mean	R%*
BhIP	$6.5±0.4^{A}$	$3.8±0.3^{B}$	41.5	$3.1±0.2^{D}$	52.3
MelQx	$0.7±0.1^{A}$	$0.5±0.1^{B}$	28.6	$0.4±0.2^{D}$	42.9
4,8-DiMelQx	$1.9±0.1^{A}$	$1.2±0.1^{B}$	36.8	$1.0±0.1^{D}$	47.4
Norharman	$3.4±0.2^{A}$	$1.8±0.1^{B}$	47.1	$1.5±0.1^{D}$	55.9

Significant differences are indicated by different superscripts in the same column (P<0.05).
BHT: Butylated Hydroxyl Toluene

DISCUSSION

Findings in Table, which indicated that 4,8-DiMelQx and IQ were UDL, are in line with those of Oz and Kaya (2011), who found that meatballs from various parts of Turkey had an average total HAA concentration of 5.54 ng/g. Furthermore, samples obtained from Samsun, Turkey, had the maximum amount of total heavy metals (7.73 ng/g). Busquets *et al.* (2004) discovered that in some home-cooked meat dishes from Spain, 4,8-DiMeIQx was less than 1.8 ng/g.

BhIP, MelQx, and Norharman were 0.54 ± 0.02, 0.25 ± 0.01, and 0.13 ± 0.01 µg/kg, according to the results listed in Table 2. Despite being UDL, 4,8-DiMelQx and IQ are in line with the findings of Margit *et al.* (2013), who found that 4,8-DiMeIQx was found in trace amounts in all Danish home-grilled meat varieties, and Kizil and Oz (2011), who proposed that harman and norharman, two aminocarbolines, were primarily formed above 300 °C.

Results in Table 2 indicate that the estimated carcinogenic HAA level (µg/kg) of the samples of charcoal-roasted kofta (n=30) that were examined were 15.39 ± 1.05, 2.08 ± 0.16, 3.73 ± 0.31, 9.22 ± 0.67 and 0.35 ± 0.02 ± 0.02 µg/kg for MelQxhar, BhIP, 4M, and 8.22 ± 0.67 and 0.35 was Norharman, while IQ was UDL. These findings are in line with Pais and Knize's (2000) findings that at moderate temperatures, more MeIQx was produced than PhIP, but PhIP was dominant in the well. -prepared samples These findings aligned with previous research indicating reduced MeAαC and comparatively elevated AαC levels in cooked meat and barbecue sauce (Turesky *et al.*, 2005).

Furthermore, Dong *et al.* (2011) mentioned that a sample of chicken breast and ground beef patties cooked in various Korean cooking conditions had no IQ.

The findings in Table 2 showed the effect of antioxidants (BOD 0.02% and carvacrol 1%) on HAAs (µg/kg) in the tested samples of charcoal-grilled meat fillets. BhIP was 3.8 ± 0.3 and the percentage reduction in BHT concentration was 41.5. 0.02%, while in carvacrol 1% individuals; The BhIP was 3.1 ± 0.2 and the percentage reduction was 52.3%, these results agree with Weisburger (2005) who reported that HCA concentration was 40% lower when butylated hydroxyanisole, a synthetic antioxidant, was used. In addition, Natale *et al.* (2014) found that the synthesis of HCAs is inhibited by synthetic and other phenolic antioxidants like sesamol and epigallocatechin gallate. Similar outcomes were noted by Gibis (2007) when using minced garlic or marinades that included garlic, onion, and lemon juice to stop the production of HCAs.

On the other hand, Ahn and Grun (2006) pointed out that spicy marinades, along with red and black pepper, can work as powerful HAA formation inhibitors. Consequently, using spices may be a useful strategy to lessen the production of HAAs. According to Gray *et al.* (2000), vitamin E sprayed on the outside of fried beef patties resulted in a 71% reduction in total HCAs. Further research is necessary to determine which kinds of compounds are more effective as well as how dosage affects outcomes.

CONCLUSION

Heterocyclic Aromatic Amines are substances that cause cancer that are created when meat is cooked at high temperatures, and using antioxidants such as BHT and Carvacrol in marinades, as well as avoiding direct heat and long cooking times, can reduce these chemicals. It is highly recommended to reduce the consumption of foods rich in carcinogens and/or mutagens.

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A CONFLICT OF INTEREST

There are no conflicts of interest, according to the authors.

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محاولات لتقدير والتحكم في المستويات المتبقية من الأمينات العطرية الحلقية غير المتجانسة في منتجات اللحوم

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الأمينات العطرية الحلقية غير المتجانسة هي مواد تسبب السرطان والتي تنشأ عند طهي اللحوم في درجات حرارة عالية لذلك يجب تجنب الحرارة المباشرة وأوقات الطهي الطويلة، يجب أن نقلل من هذه المواد الكيميائية ويجب أيضا تقليل استهلاك الأطعمة الغنية بالمواد المسرطنة.

في هذا البحث استخدمنا مضادات الأكسدة مثل كارفاكرول وبوتيل هيدروكسي تولوين للتحكم في التأثيرات المسببة للسرطان الناتجة عن الطهي بدرجة حرارة عالية عن طريق تنظيم مستويات بيروكسيد الهيدروجين في اللحوم المشوية على الفحم. تم جمع إجمالي ١٢٠ عينة من منتجات اللحوم المتمثلة في الكفتة المشوية على الفحم واللحم المشوي على الفحم (٣٠ قطعة لكل منها) بشكل عشوائي من أسواق مختلفة في محافظة القليوبية بمصر. تم تعبئة كل عينة على حدة في كيس من البلاستيك ونقلها مباشرة إلى المختبر.

تم تحليل العينات المجمعة بحثًا عن الهيدروكربونات العطرية غير المتجانسة وبشكل عام، يتم تقليل كمية الهيدروكربونات العطرية متعددة الحلقات والانحلال الحراري للدهون عن طريق تقليل كمية قطرات اللحم باستخدام مضادات الأكسدة مثل بوتيل هيدروكسي تولوين (٠,٠٢%) والكارفاكرول (١%) التي تم الحصول عليها.

أظهرت النتائج التي تم الحصول عليها أن متوسط مستويات الأمينات العطرية الحلقية غير المتجانسة في عينات اللحوم المشوية على الفحم التي تركت دون علاج (العدد = ٣٠) كانت على النحو التالي؛ BhIP وMelQx و٤,٨-DiMelQx و Norharman كانوا من Norharman بينما كان IQ هو UDL . في حين أظهرت العينات المعاملة بـ BHT 0.02%) والكارفاكرول ١%) ان هذه الأمينات قد انخفضت انخافضا ملحوظا. حيث انخفضت BhIP و MelQx و ٤,٨-DiMelQx وNorharman إلى 0.3 ± 3.8 و ٠,٥ ± ٠,١ و ١,٢ ± ٠,١ و ١,٨ ± ٠,١ مع نسبة تخفيض ٤١,٥٪ و ٢٨,٦٪ و ٣٦,٨٪ و ٤٧,١٪ على التوالي لتركيز BHT 0.02٪ .بينما كان معدل انخفاض هذه الأمينات اكبر عند استخدام الكارفاكرول بتركيز ١% حيث كانت BhIP و MelQx و٤,٨ -DiMelQx وNorharman 3.1±0.2 و٠,٤±٠,٢ و١,٠±٠,١ و١,٥±٠,١ مع نسبة تخفيض ٥٢,٣% و٤٢,٩% و٤٧,٤% و٥٥,٩% على التوالي.

نستخلص من نتائج هذه الدراسة أن معالجة اللحوم المشوية على الفحم بمضادات الأكسدة أدت الي خفض تركيزات الأمينات العطرية الحلقية غير المتجانسة في العينات المفحوصة وهو ما توصي به هذه الدراسة لتقليل مخاطر التعرض لهذه الأمينات.