HETEROCYCLIC AMINES AND POLYCYCLIC AROMATIC HYDROCARBONS IN COMMERCIAL READY TO EAT MEAT PRODUCTS ON UK MARKET

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Abstract –The daily exposure of HCAs and PAHs in selected ready to eat (RTE) meat products was evaluated and the guidance to the general public and meat producers was provided. HCAs and PAHs were extracted by solid-phase extraction and analyzed by HPLC. Chargrilled chicken contained the highest amount of HCAs (37.45±4.89ng/g) and PAHs (3.11±0.49ng/g), followed by roasted bacon (HCAs 15.24±1.31ng/g, PAHs 1.75±0.17ng/g), BBQ chicken breast (HCAs 18.81±9.02ng/g, PAHs 0.04±0.03ng/g), Tikka chicken breast (HCAs 18.07±2.56 ng/g, PAHs 0.02±0.01 ng/g) and honey salmon (HCAs 17.12±5.86ng/g, PAHs 0.38±0.09ng/g). The health risk caused by exposure of HCAs was higher in BBQ chicken, tikka chicken and honey roasted salmon than other selected meat products. Based on Lifelong Average Daily Intake (LADD) of PAHs, all RTE meat samples had relatively low health risk. This study would provide useful data to assess cancer risk of processed meat products for the general public.

Key Words – proximate composition, marinating ingredients, daily exposure.

I. INTRODUCTION

Carcinogens such as heterocyclic amines (HCAs), polycyclic aromatic hydrocarbons (PAHs) and N-nitrous compounds can be formed in processed meat products. These 5 Aminoimidazoarenes (AIAs) compounds, IQ, 2-amino-3-methylimidazo[4,5-f]quinolone, MeIQ, 2-amino-3,4-methylimidazo[4,5-f]quinolone, MeIQx, 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline, DiMeIQx, 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline and PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine have been classified into human carcinogens. PAHs are hydrocarbons that contain two or more benzene rings, such as pyrene, anthracene and naphthalene. Benz[a]anthracene (BaA) and benzo[a]pyrene (BaP) are PAHs with more potent carcinogenicity [1]. With the aim of understanding relationship of red/processed meat and cancer risk, it is useful to study the impact of meat processing and ingredients on the formation of carcinogens. Ready to eat meat consumption increased nearly twice (115 to 190g consumed per person per week) from 1975-2010 because of its convenience, they can be found either in packed sandwiches or meal dishes [2]. Therefore, the main focus of this work was to determine the concentration of HCAs and PAHs in selected RTE meat product that are popular on UK's market, in order to determine the dietary exposure of carcinogens from them and provide useful guideline about meat intake for general public.

II. MATERIALS AND METHODS

Meat samples

11 RTE meat products were purchased from a local supermarket including BBQ British chicken breast slices, tikka British chicken breast slices, Chargrilled British chicken breast slices, the British smoked ham slices, British ham slices, classic roasted bacon, crispy smoked streaky bacon, sliced pork sausage, Swedish meatballs, honey roast salmon flakes and sweet chilli salmon flakes.

Proximate analysis

The moisture content was determined by drying 3g meat at 100°C for 24 hours. Samples were dried in oven for 4 h and analyzed in Soxhlet extraction system to determine the fat level [3].

Separating and analyzing HCAs and PAHs

Each 3g ground sample was blended with 12ml 1M NaOH and then transferred into an Extrelut20 column with 17g diatomaceous earth. The HCAs were eluted out by 60ml ethyl acetate, and poured into a PRS cartridge that was conditioned with 7ml ethyl acetate, whereas PAHs were eluted by 60mL of CH_2Cl_2 containing 5% toluene. The PAHs residue was re-dissolved in 1mL n-hexane eluted

by 25mL of n-hexane and 60mL of 60:40 (v/v) n-hexane–CH_2Cl_2 mixtures in a glass column with silica gel. For HCAs, the PRS cartridge was then washed by 6ml 0.1M HCl, 15ml methanol/0.1M HCl (45/55, v/v) and 2ml water, then eluted by 20ml 0.5M ammonium acetate (pH 8.5) and transferred into a C18 cartridge which was conditioned with 5ml methanol and 5ml pure water. HCAs were eluted by 1ml methanol/ammonium hydroxide (9/1, v/v). The extract of HCAs and PAHs solvent were dried under a stream of nitrogen. The HCAs were dissolved with 50µl methanol, PAHs were dissolved in 1ml acetonitrile [1, 4].

The 5 HCAs were separated by a reversed-phase Luna 5uC18column (25cm×4.6mm, 5µm) with 0.01M triethylamine pH 3.6(A) and acetonitrile (B) (HPLC). A linear gradient of solutions was carried out that 95%A decreased to 75%A and 5%B increased to 25%B in 30min at a flow rate of 1mL/min. The temperature of column was 40°C. The DAD detector was set at 252nm.

The PAHs were separated by the same column with a mobile phase (the mixture of 84% acetonitrile and 16%HPLC water) by using a HPLC (1mL/min at 40°C). Fluorescence detector was performed by applying the excitation and emission wavelength program: 280/410 nm 0-8.50min (BaA), 376/410 nm 8.50-15min (BaP). The quantity of each individual HCA and PAHs was determined from calibration curves, which were established by the standard solutions of each HCA and PAH at 0.5,5 and 50ng/mL [1, 4].

Assessment of dietary exposure of HCAs

Only PhIP was significantly associated with increased cancer risk [5], thus PhIP level was used to assess the cancer risk of HCAs associated with meat intake on daily basis. The assessment of daily exposure of HCAs from RTE meat products was calculated below:

Daily exposure of PhIP (ng/day) = daily consumption of meat (g/day) × Concentration of PhIP (ng/g) in meat.

The daily meat consumption (g/day) for adults (both genders) were based on data from the National Diet and Nutrition Survey 2008/2009 – 2009/2010 (NDNS).

Assessment of dietary exposure of PAHs

Risk assessment of dietary exposure of PAHs was carried out by calculating Lifelong Average Daily Intake (LADD) ng/kg BW/day, the equation is:

LADD = (TEQ× IR×ED) / (BW×LT),

where TEQa (toxicity equivalent) = Conc of BaA × RPV_{BaA}. + Conc of BaP × RPV_{BaP}.

The Relative potency value (RPV) of BaP is 1 and for BaA is 0.1. IR is average intake of meat in exposure duration (g/day) based on NDNS, BW is the average body weight during exposure duration (kg); and LT is the average life expectancy for carcinogen (79.3 years old for male, 83 years old for female in UK), according to Office for National Statistics (2015).

Ingestion cancer risk= LADD × CSF,

where LADD= Lifetime average daily dose (mg/kg BW/day), CSF= Cancer slope factor $(mg/kg\ BW/day)^{-1}$ [6].

Statistical analysis

3 batches of samples were purchased for each product. Measurement of HCAs, PAHs, pH and composition was determined with three replicates. All the results were analyzed by ANOVA using SPSS Statistics 21, while Duncan's multiple test was used to investigate the difference between means. Pearson's correlation was used to investigate the relationship between HCAs/PAHs and moisture/fat level. The minimum acceptable probability for difference between samples was $p<0.05$.

III. RESULTS AND DISCUSSION

Table 1 was showed individual HCAs and PAHs levels in 11 types of ready to eat meat products. The total amount of HCAs varied from 0.05 ng/g to 43.90 ng/g. Chargrilled chicken contained the most HCAs. Only BBQ chicken and honey roast salmon had all 5 types of HCAs. The dominating compounds of HCAs in RTE meat products were IQ and 4,8-DiMeIQx. The amount of total PAHs ranged from nd to 3.56ng/g. Chargrilled chicken contained the highest amount PAHs, followed by Swedish meatballs (2.36±0.33ng/g). There were no BaA and BaP detected in BBQ chicken, tikka chicken, ham and sweet chilli salmon. While both PAHs were observed in roasted bacon, crispy bacon, sausage and Swedish meat ball. BaA was not detected in tikka chicken and sweet chilli salmon, and was not quantifiable in BBQ chicken, ham and honey roasted salmon due to a very small peak area. The range of BaP in RTE meat products was from not nd to1.09ng/g, and roasted bacon contained the highest level of BaP.

Table 1: The level of HCAs and PAHs in selected meat samples (n=9)

Sample	IQ (ng/g)	MeIQ (ng/g)	MeIQx (ng/g)	4,8-DiMeIQx(ng/g)	PhIP(ng/g)	BaP (ng/g)	BaA (ng/g)
BBQ chicken	9.16±7.58^{b}	0.07±0.07^{a}	1.87±0.31^{bc}	5.46±3.50^{ab}	2.27±0.36^{a}	Nq	Nq
Tikka chicken	9.74±2.25^{b}	Nd	Nd	2.88±1.27^{a}	5.39±1.54^{a}	Nq	Nd
Chargrilled chicken	22.68±1.99^{a}	2.72±0.59^{b}	2.93±1.08^{c}	9.11±1.49^{ab}	Nd	Nq	3.06±0.50^{d}
Ham	Nd	2.59±1.29^{b}	1.90±0.18^{bc}	Nd	Nd	Nd	Nq
Smoked ham	Nd	Nd	0.31±0.29^{a}	0.30±0.02^{a}	Nd	Nq	0.19±0.16^{ab}
Roasted bacon	Nd	2.64±0.75^{b}	Nd	12.61±0.92^{b}	Nd	1.09±0.11^{d}	0.66±0.07^{b}
Crispy bacon	Nd	3.39±0.37^{b}	Nd	1.83±0.08^{a}	Nd	0.71±0.12^{c}	0.37±0.09^{ab}
Pork sausage	Nd	2.87±0.19^{b}	Nd	2.32±1.13^{a}	Nd	0.21±0.03^{ab}	0.67±0.10^{b}
Swedish meatballs	Nd	2.11±0.42^{b}	Nd	7.02±7.69^{ab}	Nd	0.18±0.11^{ab}	2.18±0.22^{c}
Honey salmon	5.56±3.75^{b}	0.14±0.90^{a}	0.83±0.25^{ab}	4.88±3.80^{ab}	5.71±4.15^{a}	0.35±0.06^{b}	Nq
Sweet chilli salmon	2.09±0.19^{b}	Nd	0.30±0.52^{a}	0.47±0.44^{a}	Nd	Nd	Nd

Results with different letters in the same column are significantly different at the level $p<0.05$. Nd: Not detected.

Factors that affect the levels of HCAs and PAHs in processed meat

The composition of meat could also have an impact on the formation of HCAs and PAHs. As showed in Table 2, moisture content and total HCAs levels was negatively correlated, which implied that meat products with less water content seems to contain more HCAs. Ham and smoked ham samples with the highest moisture content (approx 75%) contained far less IQ, PhIP and total HCAs, compared with those in 3 chicken samples (approx 65%). Water content in meat products could dilute the concentration of carcinogens [7]. Moreover, it could prevent HCAs precursors moving to the food surface so that declined the concentration of carcinogens [8]. There was no significant correlation between fat content and total HCA level. Total HCAs were significantly higher in low fat chicken samples than bacon and sausage samples, which indicate that other factors such as cooking method might dominate the level of HCAs formation instead of fat content. In addition, increasing fat content to 15% could accelerate the heat penetration in order to produce more HCAs, but reduce the amount of carcinogens when the fat content was over 15%. Greater fat content could dilute the concentration of HCAs [9]. However, free radicals formed in lipid peroxidation might promote the formation of certain Maillard reaction products [10]. Total PAHs level was negatively correlated to moisture content and positively correlated to fat content. The possible mechanism could be that pyrolysis of heated fat that dropped on heating resources and deposited on the surface of meat [11].

Table 2: Correlation coefficients between total carcinogens level and moisture/fat content in meat

Components	Correlation coefficient(p)	p-value
Total HCAs/Moisture	-0.708	0.001
Total HCAs/Fat	-0.213	0.317
Total PAHs/Moisture	-0.734	0.001
Total PAHs/Fat	0.414	0.046

It is noticed that the marinating/processing ingredients may affect the formation of carcinogens. The appearance of HCAs in sweet chilli salmon (2.59 ng/g) was nearly 8 times lower than those in honey salmon (17.12 ng/g), it was supposed that red pepper, garlic, onion and paprika extract could reduce the amount of HCAs. Diallyl disulphide and dipropyl disulphide (in garlic and onion) were the most effective organosulphide compounds that declining the level of HCAs. They may contribute to trap intermediates in Maillard reaction so that prohibit further reactions, and also they may be regarded as scavengers of free radicals. PhIP level was significantly inhibited in sweet chilli salmon, comparing with in honey roast salmon. Polyphenol compounds (in red pepper) could be directly trapping phenyl acetaldehyde (a major precursor of PhIP) in order to inhibit PhIP forming [1]. However, which compounds are more effective and the dosage effect need to be further investigated.

Theoretical exposure and health impact

Relative risk of colon cancer was significantly correlated to PhIP intake; it was informed that colon cancer risk would significantly increase 47%, where the exposure of PhIP increased from

6.5ng/day to 41.4ng/day [5]. The average dietary exposure of PhIP from BBQ chicken, tikka chicken and honey roasted salmon were listed in Table 3. The median amount of HCAs daily intake for European people is 103ng/day, which was consistent with the total HCAs in this work (17ng-900ng/50g meat) [12]. Due to the highly consumption of chicken domestically, the exposure of PhIP from both chicken samples was relatively high.

Table 3: Daily exposure of PhIP in 3 RTE meat samples

	Daily exposure of PhIP[1] (ng/day)			
	Male		Female	
	19-64y	>65y	19-64y	>65y
BBQ chicken	154.36	88.53	124.85	77.18
Tikka chicken	366.52	210.21	296.45	183.26
Honey salmon	57.1	97.07	62.81	85.65

[1]Average PhIP determined in BBQ chicken: 2.27 ng/g, in Tikka chicken: 5.39 ng/g, in honey roasted salmon: 5.71ng/g.

Table 4: Lifelong Average Daily Intake (LADD) of PAHs from RTE Meat Products in UK

	LADD (ng/kg BW/day)			
	Male		Female	
	19-64y	>65y	19-64y	>65y
BBQ chicken	trace	trace	trace	trace
Tikka chicken	trace	trace	trace	trace
Chargrilled chicken	0.1924	0.1103	0.1878	0.1161
Ham	trace	trace	trace	trace
Smoked ham	0.0032	0.0030	0.0025	0.0028
Roasted bacon	0.1924	0.1817	0.1548	0.1676
Crispy bacon	0.1243	0.1174	0.1000	0.1083
Pork sausage	0.0435	0.0359	0.0247	0.0247
Swedish meatballs	0.0515	0.0368	0.0355	0.0355
Honey salmon	0.0324	0.0550	0.0429	0.0586
Sweet chilli salmon	trace	trace	trace	trace

The LADD of PAHs was estimated in Table 4. The greatest LADD was 0.1924 and 0.1817 ng/kg BW/day for men (19-64y and >65y). LADD were generally higher in men than in women, mainly because of the higher daily meat consumption in men. LADD for elderly were lower than for adults. Bacon and chargrilled chicken had higher values than other types of meat due to higher toxic potency and large amount of consumption.

IV. CONCLUSION

Proximate composition and ingredients could alter the formation of carcinogens. This work provides quantitatively to the intake guideline of HCAs and PAHs for public health.

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