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Dietary exposure to heterocyclic amines in high-temperature cooked meat and fish in Malaysia

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The intake of heterocyclic amines is influenced by the amount and type of meat and fish ingested, frequency of consumption, cooking methods, cooking temperature, and duration of cooking. In this study, the dietary intake of heterocyclic amines in Malaysia and their main sources were investigated. Forty-two samples of meat and fish were analysed by high-performance liquid chromatography with photodiode array detector to determine the concentration of the six predominant heterocyclic amines, namely: 2-amino-3-methylimidazo[4,5-f]quinoline (IQ), 2-amino-3,4-dimethylimidazo[4,5-f]quinoline(MeIQ), 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx), 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline (4,8-DiMeIQx), 2-amino-3,7,8-trimethylimidazo[4,5-f]quinoxaline (7,8-DiMeIQx), and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP). Dietary intake data were obtained using a food-frequency questionnaire when interviewing 600 Malaysian respondents. The level of total heterocyclic amines in food samples studies ranged from not detected to 38.7 ng g^{-1}. The average daily intake level of heterocyclic amine was 553.7 ng per capita day^{-1}. The intake of PhIP was the highest, followed by MeIQx and MeIQ. The results reveal that fried and grilled chicken were the major dietary source of heterocyclic amines in Malaysia. However, the heterocyclic amine intake by the Malaysian population was lower than those reported from other regions.

Keywords: exposure assessment; heterocyclic amines; cooked foods

Introduction

Heterocyclic amines (HCAs) are possible human and animal carcinogens formed in cooked meat and fish during high-temperature cooking processes (International Agency for Research on Cancer (IARC) 1993). The IARC also reported that HCAs such as 2-amino-3,4-dimethylimidazo[4,5-f]quinoline, (MeIQ), 2-amino-3,8-dimethylimdazo[4,5-f]quinoxaline (MeIQx), and 2-amino-1-methyl-6-phenylimidazo [4,5-b] pyridine (PhIP) are possible human carcinogens; and 2-amino-3-methylimidazo[4,5-f]-quinoline (IQ) as a probable carcinogen. HCAs also cause cancer at a variety of multiple sites in laboratory animal species (El-Bayoumy et al. 1995). Several case-control studies in various populations have reported positive associations between a higher intake of well-cooked meat and the risk of colon cancer (Sinha et al. 1999), breast cancer (Sinha et al. 2000), lung cancer (Sinha 1998), and gastric cancer (de Stefani et al. 1998).

Humans are exposed to potentially toxic substances including naturally occurring mutagenic and carcinogenic compounds. One's daily diet may contain HCAs and polycyclic aromatic hydrocarbons (PAHs) as a result of high-temperature processing of meat and fish.

Both groups are easily found in grilled and fried meat, poultry and fish (Gomaa et al. 1993; Kazerouni et al. 2001; Jägerstad and Skog 2005). The present study is focused on HCAs. Friedman (1996) reported that some HCAs play an important role in the taste, flavour, and even smell of heated meat. HCAs are formed by the pyrolysis of protein and amino acids in protein-rich foods. Their formation depends on several factors such as temperature, cooking method and time, the degree of doneness, the concentration of HCA precursors, and the presence of compounds with enhancing or inhibitory effects (Felton et al. 2000; Busquets et al. 2004). There has been a large variability of estimated intake of dietary HCAs reported from different countries (such as Japan, Singapore, Sweden, Switzerland, Spain, the United Kingdom, and the United States). The reason may be due to different cooking methods, cooking temperature, duration of cooking, different questionnaire make-up, and the studied populations. However, for the investigation of human carcinogens, an accurate assessment of the individual consumption of HCAs is essential. Up-to-date, extensive research on HCAs has been performed on the Western diet (Wakabayashi et al. 1992; Skog 1993; Knize et al. 1995; Zimmerli et al. 2001; Skog and Solyakov 2002;

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Cantwell et al. 2004; Llobet et al. 2006; Domingo et al. 2007; Martí-Cid et al. 2007). Fried meat is an important source of exposure to HCAs in the Western diet. Although HCAs are formed at ppb levels (ng g⁻¹), their intake may be dangerous for human health because of the total amount of cooked meat or fish consumed (Busquets et al. 2004).

This is the first extensive study on HCA exposure in Malaysia. The analysis of Malaysian foods for the occurrence of HCAs is important because there is worldwide human exposure to these compounds according to many reports (Augustsson et al. 1997; Byrne et al. 1998; Kobayashi et al. 2002; Wong et al. 2005). The aim of the present study was to determine the level of major six HCAs – IQ, MeIQ, MeIQx, 4, 8-DiMeIQx, 7, 8-DiMeIQx and PhIP – in some of the most commonly consumed foods in Malaysia in order to estimate the exposure of the Malaysian population to these toxic compounds. The assessment was performed by combining the content of HCAs in cooked food samples analysed in the laboratory with information on food consumption from the food frequency questionnaire.

Materials and methods

Study population and questionnaire

A cross-sectional survey was conducted in the central zone of Peninsular Malaysia covering three locations: Kuala Lumpur, Putra Jaya, and Selangor state. The respondents comprised three main races: Malay (59.5%), Chinese (29.8%), and Indian (10.2%), and other ethnic (0.5%), which were almost similar in proportion to the Malaysian population. A total sample size of 600 adult respondents aged 18 years and above was selected randomly for the survey. One adult was chosen to represent the pattern of the whole household. The sample size was measured by using a two-staged stratified random sampling method and the following equation was applied in the sample size estimation:

$$N = \frac{1.96^2 \times P(1 - P)}{d^2} \qquad (1)$$

where a Z-value of 1.96 for $p = 0.05$ (95% confidence level); P is the estimated prevalence of the sample equal to 0.5; and d is a margin of error of 0.1 for ±10%.

In the first stage of stratified random sampling, 75 enumeration blocks were selected from living quarters where each living quarter consists of between 80 and 120 houses. In the second stage of stratified random sampling, eight households were selected from each enumeration block. The enumeration blocks and living quarters for the survey were provided by the Department of Statistics Malaysia.

Study design and questionnaire

The questionnaire was divided into three sections: cooking utensils and cooking methods used at home; demographic data of the respondent; and dietary intake. Dietary intake was assessed using a food frequency questionnaire with a 1-month recall. The food frequency questionnaire included information on food items, the frequency of food consumption and the portion size of food ingested. Before the main survey, a pilot survey with 60 respondents was performed in the targeted areas to test question planning in the questionnaire.

Selection of food items and sampling of foods

Food items were selected based on the literature published in peer-reviewed journals, which included all types of processed (such as drying and smoking) and cooked (such as grilling, roasting and frying) foods reported to contain HCAs. There were in total 42 types of food items; three samples were purchased from the same stall at different times of the day. The selected meat and fish dishes for this study were fried fish, fried chicken (deep-fried), chicken soup, chicken curry, steamed fish, chicken cooked with chilli (masak merah), chicken burger, fish curry, fried chicken, keropok lekor, chicken satay, chicken randang, fish fried in chilli, nugget, fish in tamarind (asam pedas ikan), paprika chicken, sweet-and-sour fish, sausage, fish soup, chicken in coconut milk gravy (ayam masak lemak), fried salted fish, fish in coconut milk, chicken percik (ayam percik), fried beef, beef cooked with chilli, roasted fish, beef curry, smoke fish, fish in soya sauce, beef burger, beef satay, beef rendang, chicken golek (ayam golek), fish in taucu sauce, beef soup, chicken kurma, mutton satay, paprika beef, fish with three flavours, grilled chicken tandoori, mutton curry, beef in coconut milk gravy (masak lemak daging), barbecue-grilled chicken, black pepper-grilled chicken, beef kurma, and honey-grilled chicken.

Samples of all food items were purchased from local food stalls in Kuala Lumpur, Selangor, and Putrajaya. Upon arrival in the laboratory, samples' bones were removed from the meat and fish, then samples were homogenized using a Waring blender (Dawsonville, GA, USA). The homogenized samples were then pooled and mixed together before being quarter sampled. The representative samples were stored at −18°C until analysis.

Statistical analysis

Statistical analyses were performed using SPSS (version 14.0). The reliability analysis was measures as an internal consistency. Cronbach's alpha was the measurement for internal consistency. The survey was

considered to be reliable if Cronbach's alpha was at least 0.7. The descriptive analysis, including the mean, median, mode, standard deviation, and frequency of food taken by respondents, was used to describe the basic features of the data collected from the survey. An independent sample *t*-test was used to compare the differences between two genders. Analysis of variance (ANOVA) was used to test the differences between different HCAs in different food items. A $p < 0.05$ was considered to be statistically significant.

Chemicals

Solvent and chemicals were of both high-performance liquid chromatography (HPLC) and analytical grade. The water was purified in an Elix-MilliQ system (Millipore, Bedford, MA, USA). All solutions were passed through a 0.45-µm filter before injection into the HPLC system. HCAs standards, i.e. 2-amino-3-methylimidazo[4,5-f]quinoline (IQ), 2-amino-3,4-dimethylimidazo[4,5-f]quinoline (MeIQ), 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx), 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline (4,8-DiMeIQx), 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), 2-amino-3,7,8-trimethylimidazo[4,5-f]qunoxaline (7,8-DiMeIQx), and 2-amino-3,4,7,8-tetramethylimidazo[4,5-f]quinoxaline (4,7,8-TriMeIQx) as internal standard, were purchased from Toronto Research Chemicals (Downsview, ON, Canada). Empty Extrelut-20 extraction cartridges were provided by Merck (Darmstadt, Germany) and Oasis MCX cartridges (3 ml/60 mg) by Waters (Milford, MA, USA). MCX cartridges were pre-conditioned with ethyl acetate (2 ml). The solute diatomaceous earth refill material was obtained from IST (Hengoed, UK).

Determination of LOD and LOQ

The limit of detection (LOD) and limit of quantification (LOQ) were obtained by performing seven determinations of the lowest acceptable standard concentration. LOD was calculated as three times the standard of deviation (SD); LOQ was ten times the SD of the average background signal.

Recovery test

Recovery assays were established by spiking known amounts of the matrices (200 and 500 ng g^{-1} sample) of the HCA standard solution to samples. Seven replicates were conducted on each fortified sample. The recovery assays were calculated by comparing the concentration of the fortified samples with those of the unfortified samples.

HCA extraction

The extraction and clean-up of all six HCAs studied was carried out following the modified method of Gross and Grüter (1992) and Messner and Murkovic (2004). Samples (1 g) spiked with internal standard (4,7,8-TriMeIQx, 250 ppb) were dissolved in 12 ml of 1 M NaOH and the suspension was homogenized using magnetic stirring for 3 h with 500 rpm (Heidolph, MR 3001 K). The alkaline solution was mixed with diatomaceous earth (13 g), and then transferred into empty Extrelut columns. Ethyl acetate was used as the extraction solvent and elute was passed through the coupled Oasis MCX cartridges. The MCX cartridges were washed with 0.1 M HCl (2 ml) and MeOH (2 ml). The analytes were eluted with 2 ml of MeOH-concentrated ammonia (19/1, v/v). The samples were evaporated to dryness under a stream of nitrogen and the final extracts were dissolved in 100 µl methanol just before analysis for HCAs. Recovery of HCAs was determined using the standards addition method. The standards mixture (200 and 500 ng g^{-1} sample) was spiked to the chicken, beef, and fish samples before the extraction step. HCAs were separated using reversed-phase LC and a TSK gel ODS T_M column (25 cm × 4.6 mm, 5 µm). The LC systems consisted of a Waters LC model 2690 (CA, USA) with a photo-diode array detector (Waters model 2996), equipped with a quaternary solvent delivery system under a Waters Controller model 600, an autosampler (Waters model 717), and an on-line vacuum degasser. The mobile phase consisted of solvent A, 0.01 M triethylamine in water (adjusted to pH 3.3 with acetic acid), and solvent B, acetonitrile (HPLC grade; Merck). Analyses were carried out in a gradient mode for 33 min. Gradient programming was performed as follows: at zero to 18 min it was 5% to 23% B in A, at 18–25 min it was 23% B in A, and at 25–33 min it was 23% to 25% B in A. The flow rate of the mobile phase was 1 ml min^{-1}.

Estimated daily intake

The estimated daily intake levels of HCAs by an adult (ng day^{-1} per capita) were calculated using the average of each meat and fish product, i.e. (Hajeb et al. 2009):

Daily intake per person per day]

$= $ mean concentration (ng g^{-1}) of HCAs × amount of

meat and fish products consumed per day (g per capita)

Results and discussion

The socio-demographic characteristics of the study population are summarized in Table 1. The distribution for the gender and the ethnic group of the respondents represented the Malaysian population.

Table 1. Socio-demographic characteristics of respondents.

Demographic profile	Frequency	%
Gender		
Male	303	50.5
Female	297	49.5
Ethnicity		
Malay	357	62.5
Chinese	179	29.8
Indian	61	10.2
Others	3	0.5
Age group (years)		
18–24	130	22.2
25–29	91	15.2
30–34	64	10.7
35–39	64	10.7
40–44	67	11.2
45–49	52	8.7
50–54	48	8
55–59	30	5
60–64	23	3.8
65–69	20	3.3
≥70	11	1.8
Education level		
Uneducated	21	3.5
Primary school	74	12.3
Secondary school	280	46.7
College	105	17.5
Bachelor degree	80	13.3
Postgraduate	14	2.3
Others	20	3.3
Career categories		
Government servant	56	9.3
Private sector	185	30.8
Self-employed	78	13
Farmer	1	0.2
Student	96	16
Housewife	129	21.5
Others	55	8.3
Household income		
≤1000	101	16.8
1000–1999	131	21.8
2000–2999	140	23.3
3000–3999	79	13.2
4000–4999	61	10.2
≥5000	88	14.7
Frequency of eating out		
Everyday	104	17.3
Once a week	78	13
Two to three times a week	133	22.2
Two to three times a month	102	17
Once a month	136	22.7
Others	47	7.8
Cooking utensils		
Stove	592	98.7
Microwave	265	44.2
Oven	221	36.8
Grilled-stove	80	13.3
Grilled charcoal	64	10.7
Others	23	3.8

(continued)

Table 1. Continued.

Demographic profile	Frequency	%
Cooking method		
Stir-fried	394	65.7
Deep-fried	248	41.3
Boiling	204	34
Baking	200	33.3
Grilled	249	41.5
Roasted	255	42.5

Table 2. Reliability summary of food frequency questionnaire.

Variable	Cronbach's Alpha
Food consumption frequency	0.730
Food consumption portion size	0.634
Buy or cook the foods	0.902
Places of food availability	0.840

The percentage for male respondents was 50.5%; for female respondents it was 49.5%. There were 59.5% Malay respondents, 29.8% Chinese, 10.2% Indian, and 0.5% of other ethnic (Table 1). Overall, the cooking method/apparatus of respondents was mostly by stove (98.7%), followed by microwave (44.2%) and oven (36.8%). The higher usage frequency of stove, microwave and oven was probably due to their convenience. A grilled stove (13.3%) and grilled charcoal (10.7%) were used less by respondents. On the other hand, the mostly used type of cooking was stir-fried (65.7%) followed by deep-fried (41.3%), boiling (34%) and baking (33.3%). This fact showed that respondents preferred to consume fried foods (stir-fried and deep-fried) rather than other types of food (Table 1). HCAs are toxic compounds formed during grilling and frying of meat products in oil (Jägerstad et al. 1998). The grilling/barbecuing method of food preparation has been shown to produce significant level of HCAs (Keating and Bogen 2004). In the United States, Sinha et al. (1995) reported the highest level of PhIP in pan-fried or grilled/barbecued chicken samples. In that study, grilled/barbecued chicken contained the highest levels of PhIP ranging from 27 to $480 \, ng \, g^{-1}$ and pan-fried chicken contained PhIP ranging from 12 to $70 \, ng \, g^{-1}$. In other studies in the United States, Knize et al. (1997) also reported high level of PhIP ($270 \, ng \, g^{-1}$) in grilled chicken, whereas Keating and Bogen (2004) reported PhIP level of $38.2 \, ng \, g^{-1}$ in fried chicken and $327.6 \, ng \, g^{-1}$ in grilled chicken. In Spain, Busquets et al. (2004) reported the highest level of PhIP ($46.9 \, ng \, g^{-1}$) in fried chicken, whereas in Japan, Wakabayashi et al. (1993) PhIP was in fried codfish ($69.2 \, ng \, g^{-1}$).
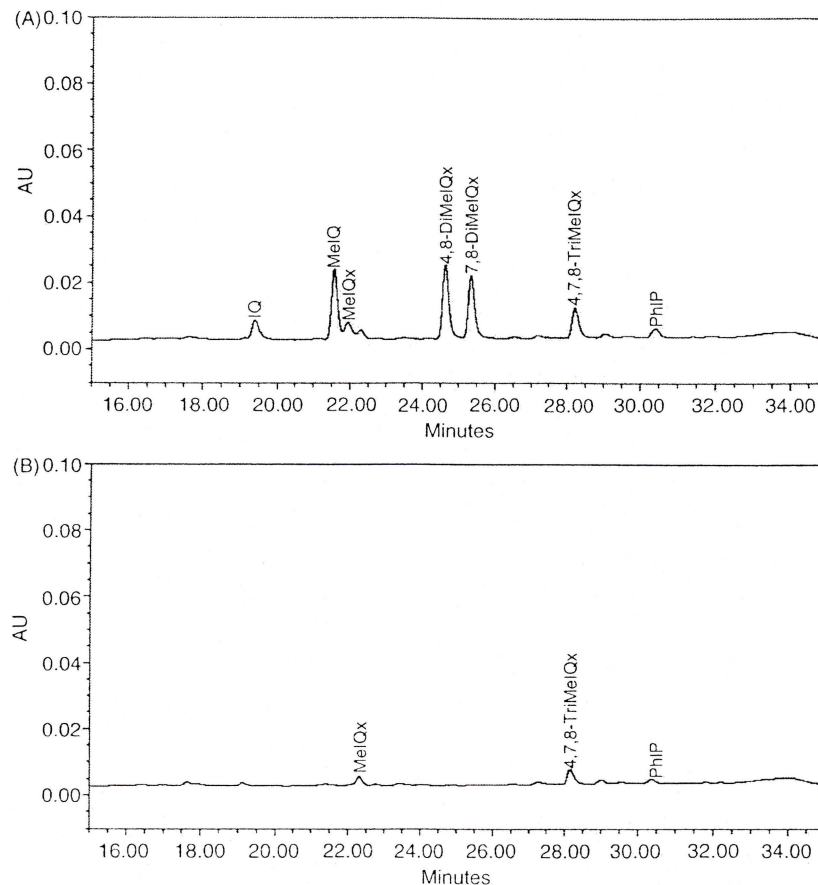
Figure 1. Representative HPLC-PDA chromatogram of a grilled chicken satay sample: (A) spiked and (B) unspiked. Results for this sample were MeIQx $= 8.1\,ng\,g^{-1}$ and PhIP $= 30.6\,ng\,g^{-1}$. Peaks were detected at 265 nm.

Reliability was used to measure the internal consistency of the study. It was measured based on the numerical coefficient of reliability, which was Cronbach's alpha (Table 2). The results given in Table 2 showed that the highest values of Cronbach's alpha were 0.902 and 0.840 for methods and for places of food availability, respectively. These were followed by 0.730 and 0.634 for food consumption frequency and portion size, respectively. In this study, all values of Cronbach's alpha were ≥0.6 where they were all accepted in exploratory factor analysis and also supported the reasonable internal consistency of variables. Therefore, the results yielded satisfactory internal consistency (coefficient alpha) reliabilities for the related variables.

The results of the food-frequency questionnaire revealed that the most popular food was fried fish (72.83%) and deep-fried chicken (68.67%), followed by chicken soup (57.67%) and chicken curry (56.50%). These findings showed that fish and chicken were the food items of choice for most respondents and that they may impose a risk of contaminants from high-temperature cooked foods.

The sample clean-up by tandem SPE using Extrelut and Oasis MCX was successful and yielded one fraction of HCAs, which was subjected to further determination by HPLC. Furthermore, the applied HPLC method provided a good separation of HCAs that make the quantification of each HCA clearly possible. Figure 1 shows a representative chromatogram on the basis of identification and quantification of individual HCA compounds. The identification of the HCA compounds found in a sample is based on their retention times and UV spectra. Figure 2 shows the UV spectra of the six authentic HCAs in sample spiked by HCA standards. All spectra observed in sample analysis were in good concordance with the spectra of the authentic compounds.

The detection limits of HCAs were: IQ, $1.5\,ng\,g^{-1}$; MeIQ, $0.5\,ng\,g^{-1}$; MeIQx, $0.3\,ng\,g^{-1}$; 4,8-DiMeIQx, $0.5\,ng\,g^{-1}$; 7,8-DiMeIQx, $0.9\,ng\,g^{-1}$; and PhIP, $1.5\,ng\,g^{-1}$, respectively. The recovery results were more than 70% for all compounds (MeIQx, 140%; 4,8-DiMeIQx, 79%; 7,8-DiMeIQx, 89%, PhIP, 75%) except for IQ (41%) and MeIQ (20%). Good
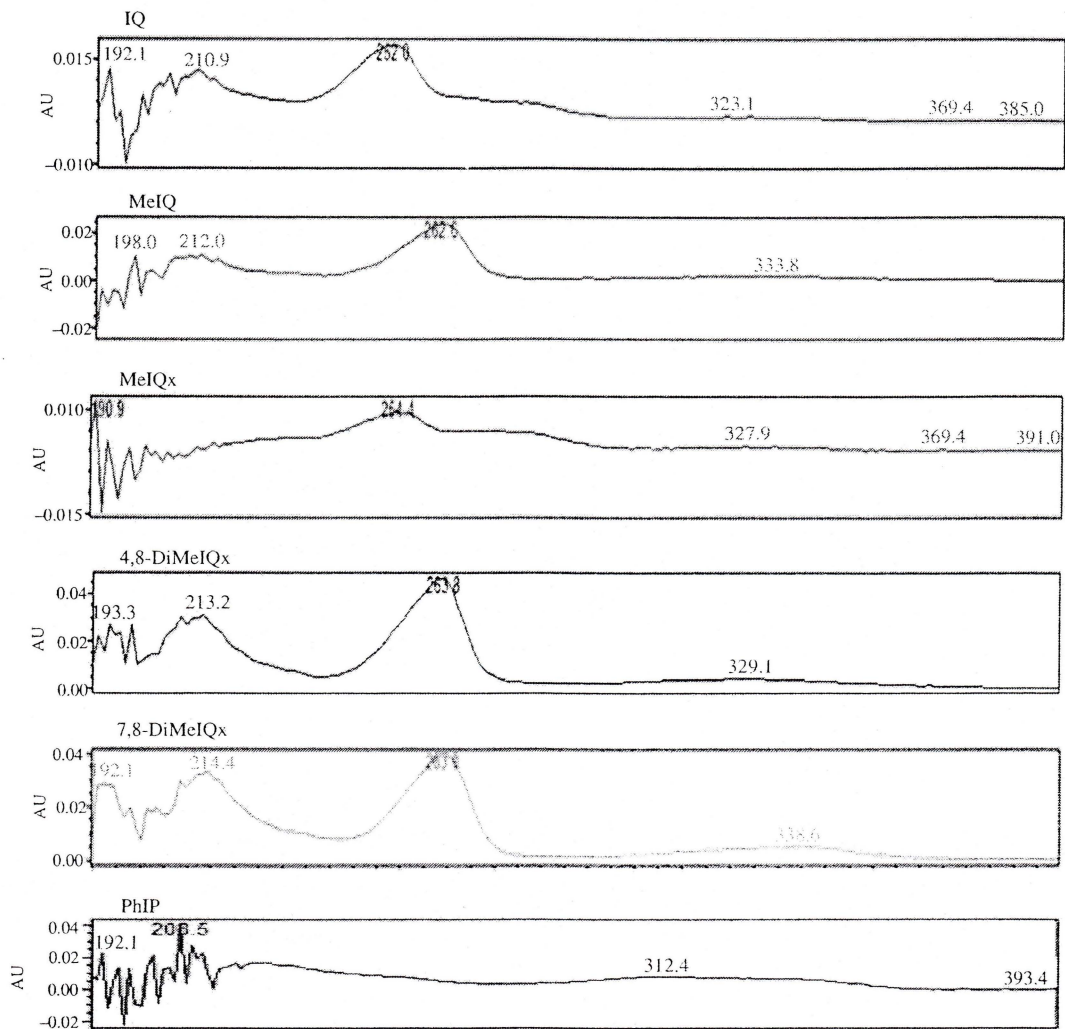
Figure 2. UV spectra of HCA standards found in spiked samples.

recoveries of spiked samples demonstrated the accuracy of the methods used in the study.

Table 3 shows the six HCAs detected in the analysed food samples. There was a significant difference ($p < 0.05$) in the amount of the six HCAs between the different foods studied. PhIP was the most commonly found HCA (24%), followed by MeIQx (23%) and MeIQ (12%) in the studied food items. Very low levels of 4,8-DiMeIQx and 7,8-DiMeIQx were detected in the samples. The highest level of total HCAs was found in grilled and fried dishes, i.e. grilled chicken satay (38.7 ng g^{-1}), followed by grilled chicken black pepper (24.3 ng g^{-1}), grilled chicken percik (24.1 ng g^{-1}) and stir-fried chicken (23.4 ng g^{-1}). The higher levels of HCAs found in grilled chicken in this study may be due to the cooking methods, temperature and cooking time, and also the precursors such as amino acid, sugar, creatinine and moisture content of the chicken. The high level of HCAs in grilled and fried foods are probably because these foods were cooked using high temperature of 125–250°C (Busquets et al. 2004) and for relatively long periods of 20–40 min (Keating and Bogen 2004). Therefore, a more frequent usage of the stove for frying may expose people to higher concentrations of HCAs. In the present study, the food samples for analysis were purchased from different restaurant. At a local food stall or restaurant there could be a significant variation in cooking time, as well as the temperature of meat and fish products sold between one restaurant and another.

Exposures vary among individuals, since dietary preferences and variation in food preparation can greatly influence individual exposure. In the Malaysian diet, meat and fish-based foods are commonly consumed. From the present study, Malaysians who are

Table 3. HCAs concentrations in meat and fish samples (ng g^{-1}).

Food item	Cooking method	IQ	MeIQ	MeIQx	4,8-DiMeIQx	7,8-DiMeIQx	PhIP	Total
Chicken curry	Boiled	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Chicken soup	Boiled	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Chicken in milk gravy	Boiled	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Chicken percik	Grilled	n.d.	n.d.	14.5	n.d.	n.d.	9.6	24.1
Chicken kurma	Fried cooked	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Fried chicken	Stir fried	n.d.	n.d.	23.4	n.d.	n.d.	n.d.	23.4
Chicken cooked with chilli	Fried/cooked	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Chicken honey	Grilled	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Chicken paprika	Fried/cooked	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Chicken black pepper	Grilled	n.d.	n.d.	24.3	n.d.	n.d.	n.d.	24.3
Chicken barbecue	Grilled	n.d.	n.d.	4.2	n.d.	1.6	9.9	15.7
Chicken tandoori	Grilled	n.d.	n.d.	n.d.	n.d.	n.d.	4.4	4.4
Chicken burger	Fried	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Chicken rendang	Boiled	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Chicken satay	Grilled	n.d.	n.d.	8.1	n.d.	n.d.	30.6	38.7
Chicken golek	Boiled	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Sausage	Fried	n.d.	n.d.	5.5	n.d.	n.d.	n.d.	5.5
Nugget	Fried	n.d.	n.d.	n.d.	n.d.	1.5	11.1	12.6
Fish with three flavour	Boiled	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Fish in milk gravy	Boiled	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Fish curry	Boiled	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Fish soup	Boiled	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Salted fish	Fried	n.d.	2.4	n.d.	n.d.	n.d.	4.8	7.2
Fish in tamarind	Fried/cooked	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Fried fish in chilli	Fried/cooked	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Sweet sour fish	Fried/cooked	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Fried fish	Deep fried	n.d.	1.4	8.8	n.d.	n.d.	n.d.	10.2
Fish in taucu sauce	Fried/cooked	n.d.	3.8	n.d.	n.d.	1.6	n.d.	5.4
Fish in soya sauce	Fried/cooked	n.d.	3.3	n.d.	n.d.	n.d.	n.d.	3.3
Steam fish	Steam	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Charcoal grill fish	Grilled	n.d.	1.6	1.7	0.5	0.9	8.4	13.1
Keropok lekor	Fried	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Beef satay	Grilled	n.d.	n.d.	n.d.	0.7	n.d.	13.3	14.0
Beef kurma	Boiled	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Beef paprika	Boiled	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Beef curry	Boiled	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Beef burger	Fried	n.d.	n.d.	n.d.	n.d.	1.0	15.1	16.1
Beef rendang	Fried/cooked	n.d.	n.d.	12.3	0.7	n.d.	n.d.	13.0
Beef soup	Boiled	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Beef in coconut milk	Boiled	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Mutton satay	Grilled	n.d.	n.d.	1.5	n.d.	n.d.	14.2	15.7
Mutton curry	Boiled	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Notes: n.d.: Not detected.
Values below the limit of detection (LOD) are calculated as 0 ng g^{-1}. LODs of: IQ = 1.5 ng g^{-1}; MeIQ = 0.5 ng g^{-1}; MeIQx = 0.3 ng g^{-1}; 4,8-DiMeIQx = 0.5 ng g^{-1}; 7,8-DiMeIQx = 0.9 ng g^{-1}; and PhIP = 1.5 ng g^{-1}.

middle-aged (18–34) or older (more than 55) generally consume more meat and fish compared with other groups of respondents. On average, Malaysians consumed 104.16 g of meat and fish dishes per day. Wong et al. (2005) reported the average daily intake of 108.7 g day^{-1} for all meat types in Singapore. Other population-based surveys have reported the daily consumption of meat from 80 to 160 g day^{-1} in the United States, Sweden, New Zealand, and Japan (Augustsson et al. 1997; Byrne et al. 1998; Kobayashi et al. 2002; Ferguson 2002). Data from the present study show that foods contributing to the intake of HCAs in Malaysia are similar to those found in Western countries.

The daily intake of HCAs from meat and fish dishes was measured for the Malaysian population (Table 4). It was found that the respondents are exposed to dietary HCAs that mostly are derived from meat. The average daily intake of HCAs was 553.7 ng per capita day^{-1}. There were relatively few numbers of specific meat and fish dishes that accounted for a large contribution of this exposure. Table 5 shows a summary of the literature reporting HCAs in foods from different regions. The results for the intake of HCAs

Table 4. Mean daily intake of HCAs from meat and fish (ng per capita day^{-1}) in Selangor, Malaysia.

Food item	Total HCAs (ng g^{-1})	Food consumption (g day^{-1})	Exposure (ng per capita day^{-1})
Chicken curry	0	2.34	0
Chicken soup	0	2.56	0
Chicken in coconut milk gravy	0	2.80	0
Chicken percik	24.1	0.08	1.9
Chicken kurma	0	0.40	0
Fried chicken	23.4	8.0	187.2
Chicken cooked with chilli	0	3.10	0
Chicken honey	0	0.4	0
Chicken paprika	0	2.34	0
Chicken black pepper	24.3	0.08	1.9
Chicken barbecue	15.7	0.20	3.1
Chicken tandoori	4.4	0.08	0.3
Chicken burger	0	3.38	0
Chicken rendang	0	2.48	0
Chicken satay	38.7	2.16	83.6
Chicken golek	0	0.18	0
Sausage	5.5	6.96	38.3
Nugget	12.6	8.10	102.1
Fish with three flavour	0	1.60	0
Fish in coconut milk	0	1.0	0
Fish curry	0	0.38	0
Fish soup	0	0.8	0
Salted fish	7.2	1.36	9.8
Fish in tamarind	0	13.28	0
Fried fish in chilli	0	10.42	0
Sweet sour fish	0	1.40	0
Fried fish	10.2	6.14	62.6
Fish in taucu sauce	5.4	1.0	5.4
Fish in soya sauce	3.3	4.22	13.9
Steam fish	0	1.10	0
Charcoal grill fish	13.1	0.38	5.00
Keropok lekor	0	1.96	0
Beef satay	14.0	0.76	10.6
Beef kurma	0	2.0	0
Beef paprika	0	0.6	0
Beef curry	0	1.50	0
Beef burger	16.1	0.70	11.3
Beef rendang	13.0	0.80	10.4
Beef soup	0	1.72	0
Beef in coconut milk gravy	0	4.0	0
Mutton satay	15.7	0.4	6.3
Mutton curry	0	1.0	0

Table 5. Review of previous studies on HCAs level in food (ng g^{-1}) from different countries.

Types of meat	Range of reported MeIQx concentration (ng g^{-1})	Range of reported PhIP concentration (ng g^{-1})	Country	Reference
Beef	0–1.5	0–6	Switzerland	Zimmerli et al. (2001)
	0–7.3	0–32	USA	Knize et al. (1994)
	0.2–1.3	0.8–2.0	USA	Keating and Bogen (2004)
	0.7–2.9	0.6–4.8	Spain	Busquets et al. (2004)
	0–7.1	0–30	USA	Sinha et al. (1998)
	0–1.9	0–19	USA	Felton et al. (2004)
Chicken	0–1.8	0–327.6	USA	Keating and Bogen (2004)
	0–0.3	2.3–46.9	Spain	Busquets et al. (2004)
	0–9	6–480	USA	Sinha et al. (1995)
	0	0–2.5	Switzerland	Zimmerli et al. (2001)
	0–0.6	0.02–10	Sweden	Skog et al. (1997)
Fish	<1–5	0–73	Switzerland	Gross and Grüter (1992)
	0–0.5	0–4.9	Switzerland	Zimmerli et al. (2001)
	0–0.9	<0.01–2.2	Sweden	Skog et al. (1997)

Table 6. Estimated mean daily intake of some HCAs in various countries.

Geographic location	PhIP (ng per capita day^{-1})	MeIQx (ng per capita day^{-1})	DiMeIQx (ng per capita day^{-1})	Sum of three HCAs (ng per capita day^{-1})	Total HCAs (ng per capita day^{-1})	ng kg^{-1} body mass day^{-1}	Reference
New Zealand					976[a]		Thomson et al. (1996)
Sweden	72	72	16		160[b]		Augustsson et al. (1997)
Sweden					77		Augustsson et al. (1999)
Sweden					8.53[c]		Olsson et al. (2005)
Switzerland	124–156	72–85	13–78	209–319	209–397[d]		Zimmerli et al. (2001)
USA	78.2–109.7	19.6–32.7	1.5–2.2	99.3–144.6			Sinha et al. (2001)[e]
USA	427	80.5	14.7	522.2		9.2[f]	Bogen and Keating (2001)[g]
USA	158.3–137.5	52.10–64.0	3.5–4.1	213.9–205.6			Sinha et al. (2000)[e]
USA	285.5–457.9	33–44.8	3.5–4.0	322–506.7			Byrne et al. (1998)
USA					1820	26	Layton et al. (1995)[g]
USA	160–218	93–135	6.5–10.7	259.5–363.7			Nowell et al. (2002)[e]
USA	539–1029	91–126	18.9–46.9	648.9–1201.9		11.0–19.9[h]	Keating and Bogen (2004)[g]
Japan	39–47	9.8–11.2	0.7–6.3	49.5–64.5		1.06–1.1[i]	Kobayashi et al. (2002)[g]
Japan	5–300	300–3900					Wakabayashi et al. (1997)
Germany	63	34	2	103			Rohrmann and Becker (2002)
Singapore					49.95[j]		Wong et al. (2005)
Spain	344	29	13.6–15.1	388	934[k]		Busquets et al. (2004)

Notes: [a]Intake of IQ, MeIQ, MeIQx, DiMeIQx, PhIP, AαC, MeAαC, Trp-P-1 and Trp-P-2.
[b]Intake of IQ, MeIQ, MeIQx, DiMeIQx and PhIP.
[c]IQx, MeIQx and PhIP.
[d]Sum of IQ, 7,8-DiMeIQx, MeIQ, 4,8-DiMeIQx, MeIQx, and PhIP.
[e]Mean values for control subjects and colon cancer cases.
[f]Total HCAs include IQ, MeIQx, DiMeIQx, PhIP and AαC.
[g]Based on a 70 kg body weight.
[h]Sum of PhIP, MeIQx, DiMeIQx, AαC and IQ.
[i]Total HCAs include Trp-P-1 and MeIQ.
[j]Mean intake of IQ, MeIQ, MeIQx, 4,8-DiMeIQx, 7,8-DiMeIQx, PhIP and IFP.
[k]Sum of DMIP, Glu-P-2, IQ, MeIQx, MeIQ, Glu-P-1, 7,8-DiMeIQx, 4,8-DiMeIQx, Norharman, Harman, Trp-P-2, Trp-P-1, PhIP, AαC, MeAαC.

from meat and fish were lower than those reported in the United States in 1995, where the value was 1820 ng per capita day^{-1} (Layton et al. 1995). However, our estimates of daily intake are in agreement with the studies of Keating and Bogen (2004) (1393 ng per capita day^{-1}) in the United States, and Busquets et al. (2004) (934 ng per capita day^{-1}) in Spain. However, a lower average intake of HCAs was also reported in different studies from Sweden, Switzerland, and Japan, where 160 ng per capita day^{-1} (Augustsson et al. 1997), 397 ng per capita day^{-1} (Zimmerli et al. 2001), and 77 ng per capita day^{-1} (Kobayashi et al. 2002) were estimated, respectively. Table 6 shows the estimated mean daily intakes of some HCAs in various countries. Due to differences in study design, intake frequency, cooking method, and the duration of cooking, it is difficult to make an accurate comparison of the daily intakes of HCAs between this study and other reported data from other studies. Nevertheless, in view of the more fried and grilled meat products and the variety of cooking methods for meat and fish, the estimated intakes of HCAs in the present study were almost similar to those reported by other studies (Table 6).

This evaluation of HCA exposure showed that fried and grilled meat seem to be the largest sources of HCAs in the diet in Selangor, and chicken was the greatest source of HCAs among different types of meat. From our survey, the ratio of total chicken intake per day was found to be higher than all the other meat intakes. Moreover, the concentrations of HCAs in chicken were relatively higher than any other meat. The most important contribution of these findings is the identification of the major contributors of HCAs in the Malaysian diet. In this study, among the 42 food items analysed, only ten samples accounted for more than 90% of the total dietary exposure to HCAs in the Malaysian population. It is also noted that minor changes of food choice can have a large impact on exposure to HCAs. There was not much difference in the intake of HCAs observed between men and women; the reason is presumably because the preference for meat was almost similar between them.

Conclusion

This study has concluded that barbecuing and frying produce relatively high levels of HCAs in protein-rich food products in Malaysia. The key finding was that fried and grilled chicken are major dietary sources of the total intake of HCAs among Malaysians. On the other hand, the consumption of boiled or fried cooked foods contributes very little to HCA intake. Further studies on the estimated dietary intake of HCAs should be carried out to clarify the relationship between the intake of HCAs and the risk of cancer among Malaysians.

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