UNIVERSITI PUTRA MALAYSIA

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By

MOHD SAFZAN BIN MOHD MUKHTAR

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

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September 2009

Chairman: Jinap Selamat, PhD

Faculty: Food Science and Technology

The objectives of this study were to determine the effect of cooking method on HAs concentration of chicken and beef *satay* and the effect of marinating and different cooking conditions on HAs concentration of roasted chicken. Six common HAs were investigated: 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), 2-amino 3,4-dimethylimidazo[4,5-*f*]quinoline (MeIQ), 2-amino-3,8-dimethyl-imidazo[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), 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP). Samples were extracted using 1M NaOH and ethyl acetate and clean up on solid phase extraction (SPE) column. The samples were then analysed using high performance liquid chromatography (HPLC) equipped with photodiode-array detector (DAD). Chicken and beef *satay* were grilled to two different degrees of doneness (medium and well done). Three types of cooking method were applied

to both types of *satay* i.e. charcoal grilled (treatment A), microwave pretreatment prior to grilling (treatment B), and deep fried-microwave (treatment C). Both chicken and beef *satay* samples which undergone microwave pretreatment prior to grilling (treatment B) showed significantly ($p<0.05$) lower HAs concentration as compared to charcoal grilled *satay* (treatment A). Deep fried-microwave (treatment C) was applied to both types of *satay* as an alternative method to cook *satay* and was proven to produce lesser HAs as compared to treatments A and B in medium and well done cooked *satay*. HAs concentration were compared in marinated and unmarinated chicken before roasting. Three roasting conditions were applied to chicken i.e. 160°C for 120 min (treatment X), 180°C for 90 min (treatment Y) and 200°C for 60 min (treatment Z). The study showed that marinated chicken produced significantly ($p<0.05$) lower HAs concentration as compared to unmarinated chicken in all three different cooking treatments. Meanwhile, roasting at 160°C for 120 min (treatment X) was found to produce the lowest HAs as compared to the other two treatments, 180°C for 90 min (treatment Y) and 200°C for 60 min (treatment Z). Marinating the roasted chicken with *percik* sauce has been shown to produce the lowest HAs concentration when the samples were exposed to 200°C for 60 min (treatment Z) as compared to other marinating sauces used in this study. These results revealed that marinating chicken before roasting with various spices and herbs which contain rich amount of antioxidants may reduce significantly the formation of mutagenic / carcinogenic HAs in the products.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KESAN KAEDAH DAN KEADAAN MEMASAK TERHADAP KANDUNGAN HETEROSIKLIK AMINA DI DALAM SATAY DAN AYAM BAKAR DIPERAP

Oleh

MOHD SAFZAN BIN MOHD MUKHTAR

September 2009

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Objektif-objektif kajian ini adalah untuk menentukan kesan kaedah masakan terhadap kandungan HA di dalam sate ayam dan daging lembu dan mengkaji kesan pemerapan dan kondisi masakan yang berlainan terhadap kandungan HA di dalam ayam bakar. Enam jenis HA yang telah dikaji ialah: 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), 2-amino 3,4-dimethylimidazo[4,5*f*]quinoline (MeIQ), 2-amino-3,8-dimethyl-imidazo[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), dan 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP). Sampel telah diekstrak melalui pengekstrakan fasa pepejal (SPE) dan dianalisis menggunakan Kromatografi Cecair Berprestasi Tinggi (HPLC) yang dilengkapi dengan pengesan sinaran-fotodiod (DAD). Sate ayam dan daging lembu telah dipanggang ke dua tahap panggangan yang berbeza (sederhana masak dan masak). Tiga jenis kaedah masakan telah digunakan untuk memanggang kedua-dua jenis sate iaitu memanggang menggunakan arang (rawatan A), prarawatan dengan ketuhar gelombang mikro diikuti dengan memanggang mengunakan arang (rawatan

B), dan menggoreng diikuti pemanasan menggunakan ketuhar gelombang mikro (rawatan C). Sate ayam dan daging lembu yang dimasak dengan prarawatan ketuhar gelombang mikro sebelum memanggang (rawatan B) menunjukkan kandungan HA yang ketara ($p<0.05$) lebih rendah berbanding sate ayam dan daging lembu yang dipanggang dengan menggunakan arang (rawatan A). Rawatan C (gorengan-ketuhar gelombang mikro) telah digunakan terhadap kedua-dua jenis sate tersebut sebagai cara alternatif untuk memasak sate dan ia terbukti menghasilkan kurang HA berbanding sate-sate sederhana masak dan masak di dalam rawatan A dan B. Kandungan HA di dalam ayam bakar yang diperap telah dibandingkan dengan ayam bakar yang tidak diperap. Tiga jenis kondisi masakan telah digunakan untuk membakar ayam iaitu 160°C selama 120 min (rawatan X), 180°C selama 90 min (rawatan Y) dan 200°C selama 200 min. Kajian ini menunjukkan ayam yang diperap menghasilkan kandungan HA yang ketara ($p<0.05$) lebih rendah berbanding ayam yang tidak diperap. Disamping itu, pembakaran pada suhu 160°C selama 120 min (rawatan X) telah menghasilkan kandungan HA yang paling rendah berbanding dua rawatan yang lain iaitu pembakaran pada suhu 180°C selama 90 min (rawatan Y) dan pembakaran pada suhu 200°C selama 200 min (rawatan Z). Pemerapan ayam menggunakan sos percik telah menghasilkan kandungan HA yang paling rendah apabila dibakar pada suhu 200°C selama 60 min (rawatan Z) berbanding sos-sos perap yang lain yang digunakan dalam kajian ini. Keputusan kajian ini telah mendedahkan bahawa pemerapan daging ayam dengan pelbagai rempah dan herba yang mengandungi kadar antioksida

yang tinggi dapat mengurangkan pembentukan HA yang mutagen/karsinogen secara berkesan.

I certify that an Examination Committee has met on 15 September 2009 to conduct the final examination of Mohd Safzan Bin Mohd Mukhtar on his Master of Science thesis entitled "Effect of Cooking Methods and Conditions on Heterocyclic Amines Content in *Satay* and Roasted Marinated Chicken" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The Committee recommends that the student be awarded the relevant degree.

Members of the Examination Committee are as follows:

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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Date: 14 January 2010

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

_____

MOHD SAFZAN BIN MOHD MUKHTAR

Date: 13 November 2009

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α-tocopherol	alpha-tocopherol
β-carotene	beta-carotene
%	percentage
&	and
µg	micro gram
4,8-DiMeIQx	2-amino-3,4,8-trimethylimidazo[4,5-*f*] quinoxaline
°C	degree celcius
AαC	2-amino-9*H*-pyrido[2,3-*b*]indole
ala	alanine
arg	arginine
asn	asparagine
asp	aspartic acid
C	carbon
cys	cysteine
g	gram
gln	glutamine
glu	glutamic acid
Glu	glucose
Glu-P-1	2-amino-6-methyl-pyrido[1,2-a:3',2'-*d*]imidazole
Glu-P-2	2-amino dipyridol [1,2-a:3',2'-*d*]imidazole

gly	glycine
H	hydrogen
HAs	Heterocyclic amines
harman	1-methyl-9*H*-pyrido[3,4-*b*]indole
HCl	hydrochloric acid
his	histidine
HPLC	High performance liquid chromatography
IARC	The International Agency for Research on Cancer
ile	isoleucine
IQ	2-amino-3-methylimidazo[4,5-*f*] quinoline
i.e	“id est”, that is
kg	kilogram
leu	leucine
LOD	limit of detection
LOQ	limit of quantification
lys	lysine
MeAαC	2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole
MeIQ	2-amino3,4-dimethylimidazo[4,5-*f*] quinoline
MeIQx	2-amino-3,8-dimethyl-imidazo[4,5-*f*] quinoxaline
MeOH	methanol
met	methionine
mg	milligram

min	minute
ml	mililiter
N	nitrogen
ND	not detected
ng	nano gram
norharman	9*H*-pyrido[3,4-*b*]indole
O_2	oxygen
Trp-P-1	3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole
Trp-P-2	3-amino-1-dimethyl-5*H*-pyrido[4,3-*b*]indole
phe	phenylalanine
PhlP	2-amino-1-methyl-6-phenylimidazo[4,5-*b*] pyridine
pro	proline
s	second
ser	serine
thr	threonine
trp	tryptophan
tyr	tyrosine
val	valine
v/v	volume/volume
W	watt

CHAPTER 1

INTRODUCTION

1.0 Introduction

Heterocyclic amines (HAs) are commonly found in meat and fish products cooked at temperatures greater than 150°C. These compounds are classified into two categories, pyrolytic mutagens and thermic mutagens, based on the temperature of formation. Pyrolitic mutagens are formed when proteins and/or amino acids are heated to high temperatures (>300°C) and characterized by pyridine ring with an amino group attached (Skog 1998; Wakabayashi and Sugimura 1998). Thermic mutagens are formed at lower temperatures (<300°C), with several being identified in cooked muscle foods. These compounds, also called aminoimidazoazaarenes, can be broken down into four major categories: quinolines, quinoxalines, pyridines, and furopyridines. The most commonly found HAs in foods are IQ (2-amino-3-methylimidazo[4,5-*f*]quinoline); MeIQ (2-amino3,4-dimethylimidazo[4,5-*f*]quinoline); MeIQx (2-amino-3,8-dimethyl-imidazo[4,5-*f*]quinoxaline); 4,8-DiMeIQx (2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline); and PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine) (Skog 1993; Wakabayashi and Sugimura 1998).

Many of the HAs isolated from foods have been shown to be mutagenic by the Ames *Salmonella typhimurium* mutagenicity assay (Felton *et al.* 1997) and by

mammalian cell culture such as Chinese hamster ovarian cells (Holme *et al.* 1989). Mutagenicity varies widely among individual HAs, and has been reported as high as 661,000 revertants/µg toward *S. typhimurium* TA98. Aflatoxin B1, a well-documented carcinogen, causes only 6000 revertants/µg under the same assay conditions (Holme *et al.* 1989). It has also been reported that HAs, when added to diet, will produce carcinogenic lesions in mice and rats (Esumi *et al.* 1989). Because HAs are found in a variety of cooked foods which constitute a major dietary part of the U.S. population, they are considered to be potential risk factors for human health (Hirose *et al.* 1999).

The precursors of HAs in cooked meat products are thought to be creatine/creatinine, amino acids and sugars (Jägerstad *et al.* 1983). It has been suggested that HAs formation follows the Maillard reaction through the generation of vinylpyrazine, vinylpyridine and aldyhydes (Jägerstad *et al.* 1983). Factors influencing HAs formation include the temperature, time and method of cooking, and also the concentrations of precursors present in food (Knize *et al.* 1994b; Skog 1993).

Several approaches to reduce the formation of HAs in food systems have been suggested. Concentrations of HAs precursors in meat patties (creatine, amino acids and sugar) were reduced by microwave pretreatment of the patties before frying (Felton *et al.* 1994). Food ingredients, such as vitamin E and tea phenolic antioxidant compounds, have also been shown to reduce HAs formation in meat

(Balogh *et al.* 2000; Tikkanen *et al.* 1996; Vitaglione and Fogliano 2004; Weisburger *et al.* 1994). Addition of glucose or lactose at levels ranging from 2 to 4% will reduce the overall mutagenicity of cooked ground meat (Skog *et al.* 1992). Marinating meats before cooking will also inhibit HAs formation (Salmon *et al.* 1997).

Malaysian consumption of chicken and beef per capita has increased considerably from 1985 to 2000. According to Food Consumption Statistics of Malaysia (2003), the estimated intakes of chicken and beef for Malaysian were 31.66 and 9.47 g/day, respectively. This amount indicates high probability that Malaysians consume HAs in the level that may be harmful and can lead to cancer. Thus, Malaysians generally consume more chicken than beef, and high temperature cooking method was always employed as the cooking method in a daily basis. High temperature cooking method in Malaysia's cuisines involved grilling, roasting and deep frying. Satay and roasted chicken are good examples of food prepared using high temperature cooking method which are grilling and roasting. These foods are popular among Malaysians and usually prepared according to individual preferences. In general, satay are grilled over a charcoal fire, and then served with various spicy gravies. Meanwhile, roasted chicken with different marinating flavors are cooked in oven with a certain temperature. There are different types of marinating sauces used to marinate chicken before roasting; black pepper, percik, turmeric and salt, tandoori, and honey. Both dishes are widely consumed either during festive seasons or daily routine.

Previous study by Wu *et al.* (1997) showed that HAs were present in Malay chicken satay ranging from 7.8 ng/g to as high as 84.0 ng/g. PhIP, the most abundant HAs in cooked beef, chicken and fish, was also detected in Chinese mutton and pork satay, and Malay chicken satay. However, in that study, satay was purchased from various food stalls and the grilling style might be different. Sinha *et al.* (1995) recorded unusually high level of PhIP formation in roasted chicken using high temperature cooking practice. A study by Tikkanen *et al.* (1996) showed that, roasting chicken at high temperature (220°C) can produce high level of HAs. However, marinating before roasting can reduce HAs formation in roasted chicken (Tikkanen *et al.*, 1996).

The HAs contents in popular Malaysian foods (i.e. chicken and beef satay and roasted marinated chicken) have not been fully investigated. Therefore no database and information regarding the HAs formation in Malaysian diet is available. Hence, it is vital to develop a database on the HAs content in commonly consumed food among Malaysians.

The objectives of this research were:

1) To determine the effect of cooking method on HAs content in beef and chicken satay.