UNIVERSITI PUTRA MALAYSIA

EFFECTS OF SUGAR TYPES AND ORGANIC ACIDS USED ON HETEROCYCLIC AMINES (HCAs) FORMATION IN MARINATED CHICKEN SATAY

NUR DIYANA SYAMIM BINTI HASNOL

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By

NUR DIYANA SYAMIM BINTI HASNOL

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

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Chairman: **Prof. Jinap Selamat, PhD**
Faculty: **Food Science and Technology**

The aim of the study was first to determine the effect of different types of sugar as marinating ingredients on the formation of heterocyclic amines in grilled chicken (satay). Table sugar, brown sugar, and honey were used. Internal temperature and weight loss of the grilled chicken were measured. HCA precursors (free amino acids and sugar concentration) were determined before and after marinating. HCA were quantified using Liquid Chromatography-Mass Spectrometry (LCMS) with triple quadrupole mass analyzer. The mean internal temperature of the grilled chicken marinated with table sugar, brown sugar, and honey were 82.3°C, 82.3°C, and 82.3°C, respectively and was not significant different ($p > 0.05$) with each other. Mean percentage of weight loss of the grilled chicken was significantly ($p < 0.05$) lower for marinades with honey (35%) than with table sugar (37%), and with brown sugar (38%). There was no significant difference ($p > 0.05$) in the means between treatments and control samples for amino acids. The mean concentration of fructose and glucose in chicken samples using honey marinades was found to be 3.89 and 3.68 g/100 g, respectively, and this was significantly higher ($p < 0.05$) than with brown sugar (0.70 and 1.58 g/100 g), and with table sugar (0.65 and 1.60 g/100 g, respectively). In the present study, glucose was the only sugar detected in the control samples (0.67 g/100 g). The mean concentration of sucrose was significantly ($p < 0.05$) higher in chicken samples that were marinated with table sugar (12.8 g/100 g) than those with brown sugar (8.6 g/100 g), and with honey (0.43 g/100 g). When honey was used as one of the ingredients, substantial reductions in the concentration of MeIQ, DiMeIQx, IQ, IQx, and Norharman in grilled chicken were achieved. Moreover, a correlation study has indicated that when honey was added into the recipe, the formation of most HCA (i.e., MeIQ, DiMeIQx, IQ, IQx, Norharman, and Harman) was reduced while some other HCA (i.e., PhIP, MeIQx, and AαC) increased in concentration. In addition, table sugar showed a strong correlation in enhancing the formation of all HCA except for Norharman, Harman, and AαC. To minimize the formation of HCA, the present study recommends using honey to partially replace the table sugar for the purpose of giving a sweet taste in the marinated grilled meat. The second objective was to study the use of other alternative organic acids in formulating marinating ingredients to reduce HCA in grilled chicken (satay). Chicken breast samples were marinated with table sugar, brown sugar, and honey with the addition of organic acid (tamarind, lemon, lime, and calamansi) for 24 hrs at 4°C. The pH before and after marinating were measured. HCA concentrations before and after grilling were quantified. There was a significant difference ($p < 0.05$) in the combined dependent variables (for all HCA) among the control and marinated grilled chickens. Using lemon in marinades containing table sugar, the high concentrations of DiMeIQx significantly reduced ($p < 0.006$) from 16.5 ng/g for low concentration to 8.30 ng/g for high concentration of organic acid ingredients. Similarly, in marinades containing brown sugar, the high concentrations of DiMeIQx significantly reduced ($p < 0.006$) from 35.0 ng/g for low concentration to 16.2 ng/g for high concentration of organic acid ingredients. It was observed that for all types of sugars, the mean pH of the treated samples was lower than those in the control samples. For marinades containing table sugar, the pH of chicken samples was 5.14 for tamarind, 5.28 for lemon, 5.24 for lime, and 4.94 for calamansi: these pH were significantly lower ($p < 0.05$) than those of control samples (5.48). The reduction was 57% for IQx, for MeIQx by 82%, for IQ by 61%, for DiMeIQx by 80%, and for MeIQ by 71%. Also, when calamansi was added at high concentration in marinades containing brown sugar, substantial reduction in the concentration was high; 64% for IQx and IQ, for DiMeIQx by 70 %, except for MeIQx, in which the concentrations were increased by 44 %. The highest percentage of reductions was achieved when tamarind was added at high concentration in marinades containing honey. The concentrations were reduced for IQx by 90 %, for MeIQx by 67 %, for IQ by 81 %, for DiMeIQx by 88 %, for MeIQ by 74 % and for PhIP by 76 %. High percentage of reduction was also achieved for Norharman (63 %), and AαC (81 %). Calamansi was found to reduce HCA in marinades containing table sugar and brown sugar, whereas tamarind in marinades containing honey.

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

KESAN PERBEZAAN JENIS GULA DAN ORGANIK ASID DIGUNAKAN TERHADAP PENBENTUKAN HETEROSIKLIK AMINE (HCAs) DALAM PEMERAPAN AYAM SATE

Oleh

NUR DIYANA SYAMIM BINTI HASNOL

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Pengerusi: **Profesor Jinap Selamat, PhD**
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Tujuan kajian ini adalah untuk menentukan kesan jenis gula yang berbeza sebagai ramuan dalam pemerapan ke atas pembentukan heterosiklik amina dalam ayam panggang (sate). Gulatebu, gulaperang, dan madu telah digunakan. Suhu dalaman dan kehilangan berat sampel telah dianalisa. Prekursor HCA (asid amino bebas dan gula penurun) telah ditentukan sebelum dan selepas pemerapan. HCA telah dianalisa menggunakan *Liquid Chromatography-Mass Spectrometry (LCMS)* dengan *triple quadrupole mass analyzer*. Minimum suhu dalaman sampel yang diperap mengandungi gula tebu, gula perang, dan madu adalah 82.3 °C, 82.3 °C dan 82.3 °C, masing-masing dan tidak menunjukkan perbezaan yang signifikan ($p > 0.05$) antara satu sama lain. Minimum peratusan kehilangan berat sampel menunjukkan perbezaan yang signifikan ($p < 0.05$) dan lebih rendah untuk pemerapan mengandungi madu (35%) berbanding dengan gula tebu (37%), dan gula perang (38%). Tiada perbezaan yang signifikan ($p > 0.05$) dalam sampel rawatan dan sampel kawalan untuk asid amino. Kepekatan minimum fruktosa dan glukosa dalam sampel yang telah diperap dengan madu masing-masing adalah 3.89 dan 3.68 g/100 g, dan ini adalah lebih signifikan ($p < 0.05$) berbanding dengan gula perang (0.70 dan 1.58 g/100 g), dan dengan gula tebu (0.65 dan 1.60 g/100 g). Dalam kajian ini, glukosa adalah satu-satunya gula yang telah dikesan dalam sampel kawalan (0.67 g/100 g). Kepekatan minimum sukrosa adalah signifikan ($p < 0.05$) lebih tinggi dalam sampel yang telah diperap dengan gula tebu (12.8 g/100 g) berbanding gula perang (8.6 g/100 g), dan dengan madu (0.43 g/100 g). Analisis varians multivariat (MANOVA) mendapati perbezaan yang signifikan pemboleh ubah bersandar (semua HCA) antara sampel kawalan dan yang telah diperap. Semua jenis HCA mencapai perbezaan statistik. Pengurangan di bawah 40% telah dicapai untuk MeIQx, IQx, dan Harman (kecuali PhIP, MeIQ dan Norharman yang menunjukkan penurunan 45-76%). Pengurangan peratusan yang tinggi telah didapati apabila madu digunakan iaitu 70, 66, 78, 46 dan 73% untuk MeIQ, PhIP, DiMeIQx, IQ, dan Norharman. Pengurangan peratusan dengan menggunakan gula perang ialah 77% untuk PhIP dan 67% untuk MeIQx. Kajian korelasi telah menunjukkan bahawa dengan menggunakan madu, pembentukan MeIQ, DiMeIQx, IQ, IQx, Norharman, dan Harman telah menurun manakala PhIP, MeIQx, dan AαC telah meningkat. Objektif kedua adalah untuk

mengkaji penggunaan alternative asid organic lain sebagai bahan pemerapan untuk pengurangan HCA dalam sampel. Sampel telah diperap dengan gula, gula perang, madu dan dengan tambahan asid organik (asam jawa, lemon, limau nipis, dan limau kasturi) selama 24 jam pada 4 °C. Nilai pH sebelum dan selepas pemerapan telah dicatat. Kepekatan HCA sebelum dan selepas pemanggangan telah dianalisa. Terdapat perbezaan yang signifikan bagi pembolehubah bersandar (untuksemua HCA) antara kawalan dan sampel yang telah diperap. Dengan menggunakan limau kasturi dalam pemerapan mengandungi gulatebu, kepekatan DiMeIQx telah berkurangan ($p<0.006$) daripada 16.5 ng/g untuk kepekatan rendah sehingga 8.30 ng/g. Bagi pemerapan mengandungi gula perang, kepekatan DiMeIQx berkurangan ($p < 0.006$) daripada 35.0 ng/g untuk kepekatan yang rendah kepada 16.2 ng/g. Bagi semua jenis gula, pH minimum sampel yang dirawat adalah lebih rendah daripada sampel kawalan. Bagi pemerapan dengan gula tebu, pH kiub ayam adalah 5.14 untuk asam jawa, 5.28 untuk lemon, 5.24 limau nipis, dan 4.94 untuk limau kasturi: pH ini adalah jauh lebih rendah ($p <0.05$) berbanding dengan sampel kawalan (5.48). Pengurangan ini adalah 57% untuk IQx, 82% untuk MeIQx, 61% untuk IQ sebanyak, 80% untuk DiMeIQx, dan 71% untuk MeIQ. Apabila limau kasturi ditambah pada kepekatan yang tinggi dalam pemerapan mengandungi gulaperang, peratus pengurangan dalam kepekatan adalah tinggi, iaitu 64% untuk IQx dan IQ, serta70% untuk DiMeIQx, kecuali MeIQx, di mana kepekatan yang telah meningkat ialah sebanyak 44%. Asam jawa telah ditambah pada kepekatan yang tinggi dalam pemerapan mengandungi madu mencapai peratusan yang tinggi. Kepekatan dikurangkan untuk IQx sebanyak 90%, bagi MeIQx sebanyak 67%, bagi IQ sebanyak 81%, bagi DiMeIQx sebanyak 88%, bagi MeIQ sebanyak 74% dan untuk PhIP sebanyak 76%. Peratusan yang tinggi pengurangan juga telah dicapai untuk Norharman (63 %), dan AαC (81 %). Limau kasturi didapati telah mengurangkan HCA dalam pemerapan mengandungi gula tebu dan gula perang, manakala asam jawa dalam pemerapan mengandungi madu.

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

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DECLARATION

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LIST OF ABBREVIATIONS

%	Percentage
ng	nanogram
HCA	heterocyclic amines
IQ	2-amino-3-methylimidazo[4,5-f]-quinoline
MeIQ	2-Amino-3,4-dimethyl-3H-imidazo[4,5-f]quinoline
MeIQx	2-amino-3,8-dimethlimidazo [4,5-f]-quinoxaline
DiMeiQx	2-Amino-3,4,8-trimethyl-3H-imidazo[4,5-f]quinoxaline
PHiP	2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine
IARC	International Agency for Research on Cancer
TEA	Trietylamine
AABA	Alpha amino butyric acid
PITC	phenylisothiocynate
AαC	2-amino-9H-pyrido[2,3-b]indole
Harman	1-methyl-9H-pyrido-[4,3-b]indole
Norharman	9H-pyrido-[4,3-b]indole
Cm^3	centimetre
ppb	part per billion
G	gram
µg	microgram
°C	degree celcius
mL	mililitre
v/v	volume/volume

mg/kg	milligram/kilogram
min	minutes
w/w	weight/weight
µm	micrometer
µL	microlitre
HPLC	High Performance Liquid Chromatography
R.I.	Refractive index
N	Normality
mmHg	milimole mercury
UV	ultraviolet
NaOH	Sodium Hydroxide
M	Molar
HCl	Hydrochloric acid
ESI+	Electro Spray Ionisation
HPLC-MS/MS	High Performance Liquid Chromatography Mass Spectrometry/Mass Spectrometry
SRM	selected reaction monitoring
LOD	Recovery, limit of detection
LOQ	limit of quantification
ANOVA	analysis of variance
MANOVA	multivariate analysis of variance

CHAPTER 1

INTRODUCTION

1.1 An overview of heterocyclic amines (HCA)

Human are always exposed to potentially toxic substances including mutagenic and carcinogenic compounds, in which one of them is heterocyclic amines (HCA), that may also plays an important role in cancer development (Iwasaki, et al., 2010). HCA are one of the main groups of food carcinogens resulting from high temperature cooking. In addition, HCA are formed in parts per billion (ppb) during cooking of meat (Takashi Sugimura, Wakabayashi, Nakagama, & Nagao, 2004). Last decades, there is possible relationships between diet and cancer (Oz, Kaban, & Kaya, 2010), and about 25 HCA have been identified in cooked food especially grilled, fried, broiled meat and fish (Kerstin Skog & Solyakov, 2002). Poultry is one of the most important heat-processed protein rich food sources which are available and its products and consumption are increasing rapidly (Ruth Charrondiare, et al., 2013).

In addition, HCA is formed by the pyrolysis of protein and amino acid in protein rich foods (Jahurul, et al., 2010). Their formation most likely depends on factors such as concentration of precursors, temperatures, cooking method, time, and degree of doneness (Busquets, Bordas, Toribio, Puignou, & Galceran, 2004). The international agency for research on cancer (IARC, 1993) has classified 8 of the HCA compound (2-amino-3-4-dimethyl-imidazo[4,5-f]quinoline (MeIQ), 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx), PhIP, 2-amino-9H-pyrido[2,3-b]indole (AaC), 2-amino-3methyl-9H-pyrido{2,3-b]indole (MeAaC), 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole (Trp-P-1), 3-amino-1-methyl-5H-pyrido[4,3-b] indole (Trp-P-2) and 2-amino-6-methyldipyrido[1,2-a:3',2'-d]imidazole (Glu-P-1) as possible human carcinogens (class 2B).

Moreover, (2-amino-3-methylimidazo[4,5-f]quinoline (IQ) as a probable human carcinogen (class 2A) and it is recommended to reduce the exposure to these compounds. 1-methyl-9H-pyrido [4,3-b]indole (Harman) and 9H-pyrido-[4,3-b] indole (Norharman) are often referred as co-mutagens (Turesky, 2007). However, the co-mutagens compounds cannot be neglected because even if they are not mutagenic in Ames/Salmonella test, though it has ability to enhance the mutagenic activity for other compounds such as Trp-P-1 and Trp-P-2 (T Sugimura, Keiji, Hitoshi, & N, 2004) and has relation with neurotoxins and enzyme inhibitors (Kuhn, Muller, Groaye, & Rommelspacher, 1996).

Heterocyclic amines can be classified in two groups based on their formation process: pyrolitic mutagens and thermic mutagens. Pyrolitic mutagens are formed when amino acids are heated to high temperature (>300°C) and characterised by pyridine ring with an amino group attached (Murkovic, 2007; Skog, Johansson, & Jagerstad, 1998). Thermic mutagens are formed at lower temperatures (<300°C), with several being identified in cooked muscle foods. These compounds, also called aminoimidazoazarenes which can be broken down into four major catagories: quinolines, quinoxalines, pyridines, and furopyridines (Jahurul, et al., 2010).

Extensive research activities have been carried out to understand about the occurrence of HCA in a wide range of foods and its intake. Several epidemiological studies have been carried out to test the hypothesis between meat intake and human study (Bordas, Moyano, Puignou, & Galceran, 2004) even though it is hard to link meat consumption and intake of HCA. Despite the cooking methods, temperature, time, choosing the type of meat could be one of the important factor.

World cancer research fund and american institute for cancer (2008) has extensively studied the role of HCA compounds in colorectal cancer and reported that HCAs increased the risk of colorectal cancer. Previously, several epidemiological studies have revealed that dietary exposure to HCA is associated with an increase risk of some human cancers (Kerstin Skog & Solyakov, 2002). Nevertheless there are still many conflicting results in the association between HCA intake and cancer intake (Solyakov, Skog, & Jagerstad, 1999).

1.2 Importance of study

In Malaysia, the average intake of meat and fish was estimated to be 104.16 g/day (Ang, 2009), likewise the average daily consumption of meat and fish was estimated to range from 80 to 160 g/day in United States, Sweden, New Zealand, Japan, and Singapore (Jahurul, et al., 2010). According to Food Consumption Statistics of Malaysia (2008), the estimated intakes of chicken and beef for Malaysian were 31.66 g/day. Thus, Malaysians consume HCA in the level that may be harmful which can lead to cancer easily. It is expected that the food consumption might be the major source of dietary HCA. High temperature cooking method such as grilling, barbecuing, pan frying, and deep frying have been practiced frequently in daily basis all over the world. Malaysian delicacies; satay (grilled chicken, beef, and mutton) for example is prepared at high temperatures and hence may generate the harmful by-products (HCA, PAH and others). Previous study by Wu et al., (1997), stated that chicken satay contained HCA at the range of 7.8 to 84 ng/g and PhIP was found as the most abundant HCA Nevertheless, the samples were purchased from different stall and perhaps the different of cooking style that may influence to the data collection.

Satay is usually marinated with sugar, onion, turmeric powder, lemon grass, and salt, and then served with spicy gravies containing ground peanut or soy sauce. Marinating is one of the popular techniques used to help in tenderize and improve the flavor (Lemos, Nunes, & Viana, 1999). Normally sugar is widely used as the main ingredient in recipes during cooking. Sugar is known as important precursors that lead to the formation of HCA (Skog & Jagerstad, 1990). In addition, model systems studies using the precursors such as creatine, amino acids, and sugar (glucose, fructose, sucrose, and lactose) were found to influence the yield of the formation HCA (Manabe, Kurihara, Wada, Tohyama, & Aramaki, 1992). The concentration of HCA produced depended on the sugar used. Difference types of sugar composition (reducing sugar and sucrose) closely related to the formation of HCA. Table sugar is commonly used; however, honey and brown sugar can be used as alternative sugar to replace table sugar.

Previous study by Safzan et al. (2009), determined the effect of cooking method on HCA content in beef and chicken satays. They found the alternative cooking method can be applied in minimizing HCA formation. However, the researcher was not focusing on the effect of the marinade ingredients used. Therefore, there is lack of information regarding the HCA formation especially the role of sugar and acidic ingredients on HCA formation. Table sugar is normally used in marinating recipe; therefore this study is comparing effect of table sugar with the other types of sugar (brown sugar and honey) on the formation of HCA in marinated grilled chicken samples. The concentration of sucrose and reducing sugars (such as glucose and fructose) of the different types of sugar may have an influence on the types and concentration of HCA.

Concentration of HCA in foods may be affected by cooking method, temperature of cooking, and ingredients in marinating. Food ingredients containing vitamin E and phenolic compounds, and antioxidant been reported to reduce HCA formation in meat Weisburger et al., (1995). Natural sources such as lemon, tomatoes, and garlic have been shown to reduce the formation of HCA (Vitaglione, Monti, Ambrosino, Skog, & Fogliano, 2002). However, there is limited study on the function of other acidic ingredients in reducing HCA. In our study, tamarind, lime and calamansi are used as other alternative acidic ingredients in marinating formulation ingredients.

The objectives of the research:

1. To determine the effect of different types of sugar in marinades on the formation of HCA in satay.
2. To study the use of other alternative organic acids in formulating marinating ingredients to reduce HCA in satay.

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