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DETERMINATION OF ACRYLAMIDE IN BANANA-BASED SNACKS AND EFFECT OF DIFFERENT MATURITY STAGES ON FORMATION OF ACRYLAMIDE IN BANANA FRITTERS

GISIA DANIALI

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By

GISIA DANIALI

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

July 2010

DEDICATED TO MY BELOVED FAMILY

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

DETERMINATION OF ACRYLAMIDE IN BANANA-BASED SNACKS AND EFFECT OF DIFFERENT MATURITY STAGES ON FORMATION OF ACRYLAMIDE IN BANANA FRITTERS

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July 2010

Chairman : Jinap Selamat, PhD

Faculty : Food Science and Technology

Malaysians consume considerable amount of fried and baked banana-based snacks, which have potential amount of acrylamide content. This study was carried out to a) determine acrylamide in Malaysian banana based snacks by gas chromatography- mass spectrometry, b) to study the effect of maturity stages of banana on the formation of acrylamide in banana fritters. The modified method was based on extraction with water followed by cleanup through Oasis HLB and MCX solid-phase extraction cartridges. Then it was followed by bromination (2.5 mL, saturated bromine water treatment) of acrylamide into 2, 3-dibromopropionamide prior conversion to 2-bromopropenamide by dehydrobromination with triethylamine. The results indicated that volume of 2.5 mL bromine water was sufficient to derivatize the acrylamide. The limit of detection (LOD)

and limit of quantitation (LOQ) of the modified method were 5 and 15 µg/kg, respectively, whereas the recovery for 2.5 mL of saturated bromine water ranged from 86.6 to 105.3%. Five types of Malaysian popular fried and baked banana based snacks purchased from different local markets had acrylamide at the range from 74.0 to 7468.8 µg/kg for banana fritter (*pisang goreng*), 28.9 to 243.7 µg/kg for banana chips (*kerepek pisang*), 160.7 to 500.4 µg/kg for sweet banana chips (*kerepek pisang manis*), >5 to 154.4 µg/kg for banana cake (*kek pisang*) and 31.7 to 609.1 µg/kg for banana balls (*cekodok pisang*). Analysis of variance showed significant differences ($p < 0.05$) between acrylamide concentrations in foods from different types. The highest acrylamide content was found in the banana fritter might be related to the higher heating temperature and duration of heating time. To study the effect of maturity stages of banana on the formation of acrylamide in banana fritters, two varieties of local banana *Musa paradisiaca* variety *Awak* and *Abu* were fried before acrylamide determination. The more mature banana had significantly ($p < 0.05$) higher concentrations of reducing sugars; however, the concentrations of amino acids at different maturity stages were relatively similar ($p > 0.05$). The study indicated that reducing sugar had significant ($p < 0.05$) and strong correlation (R^2= 0.92 for Abu) and (R^2= 0.82 for Awak) with the acrylamide formation, as compared to asparagine. Concentration of acrylamide in both banana varieties enhanced with the increase of both reducing sugars (glucose and fructose). This is demonstrated that the formation of acrylamide presented a strong dependence on the concentration of reducing sugar. However this study failed to show the correlation between acrylamide formation and asparagine as its precursor.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia bagi memenuhi keperluan untuk ijazah master sains

PENENTUAN AKRILAMIDA DALAM MAKANAN RINGAN BERASASKAN PISANG DAN KESAN PERINGKAT KEMATANGAN PISANG TERHADAP PEMBENTUKAN AKRILAMIDA DALAM PISANG GORENG

Oleh

GISIA DANIALI

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Pengerusi: Jinap Selamat, PhD

Fakulti: Sains Makanan dan Teknologi

Rakyat Malaysia mengambil kuantiti yang agak banyak makanan ringan berasaskan pisang yang digoreng dan dibakar yang berpotensi mengandungi akrilamida. Kajian ini telah dijalankan untuk a) menentukan kandungan akrilamida dalam makanan ringan berasaskan pisang di Malaysia dengan menggunakan gas kromatografi- spektrometri jisim, b) untuk mengkaji kesan peringkat kematangan pisang terhadap penghasilan akrilamida dalam pisang goreng. Cara pengekstrakan diubahsuai dengan menggunakan air sebagai media pengekstrakan yang disambung dengan pembersihan melalui kartrij pengekstrakan fasa pepejal Oasis HLB dan MCX. Kemudian, ia diikuti oleh pembrominan (2.5 mL, rawatan air bromin tepu) akrilamida kepada 2, 3-

dibromopropionamida sebelum penukaran kepada 2-bromopropenamida melalui dehidrobrominasi dengan trietilamina. Keputusan menunjukkan 2.5 mL air bromin adalah mencukupi untuk menghasilkan terbitan akrilamida. Had pengesanan (LOD) dan had kuantitatif (LOQ) bagi cara yang telah diubahsuai ialah 5 dan 15 μg / kg, masing-masing, manakala dapatan semula untuk 2.5 mL air bromin tepu berjulat dari 86.6 hingga 105.3%. Lima jenis makanan ringan popular di Malaysia yang berasaskan pisang yang digoreng dan dibakar dibeli daripada pasaran tempatan berbeza menunjukkan julat pembentukan akrilamida dari 74.0 hingga 7468.8 μg / kg untuk pisang goreng, 28.9 hingga 243.7 μg / kg untuk kerepek pisang, 160.7 hingga 500.4 μg / kg untuk kerepek pisang manis, daripada >5 hingga ke 154.4 μg / kg untuk kek pisang dan 31.7 hingga 609.1 μg / kg untuk bebola pisang (cekodok pisang). Analisis varians menunjukkan perbezaan yang jelas ($p < 0.05$) antara kandungan akrilamida dalam makanan daripada pelbagai jenis makanan yang berbeza. Kandungan akrilamida tertinggi telah didapati dalam pisang goreng mungkin berhubung kait dengan suhu pemanasan yang tinggi dan tempoh waktu pemanasan yang lama. Untuk mengkaji kesan peringkat kematangan pisang terhadap pembentukan akrilamida dalam pisang goreng, dua varieti pisang tempatan iaitu *Musa paradisiaca* bervarieti Awak dan Abu digoreng sebelum penentuan akrilamida. Pisang yang lebih matang menunjukkan kandungan gula penurunan yang lebih tinggi ($p < 0.05$); tetapi, kandungan asid amino di peringkat kematangan berbeza adalah sama ($p > 0.05$). Kajian ini menunjukkan gula penurunan mempunyai kaitan jelas ($p < 0.05$) dan pertalian erat ($R^2 = 0.92$ untuk Abu) dan ($R^2 = 0.82$ untuk Awak) dengan pembentukan akrilamida, berbanding dengan asparagina. Kandungan akrilamida dalam kedua-dua varieti pisang ditingkatkan dengan peningkatan kedua-dua gula penurunan (glukosa dan fruktosa), dengan ini menunjukkan

pembentukan akrilamida bergantung kuat kepada kepekatan kandungan gula penurunan. Walau bagaimanapun, kajian ini gagal menunjukkan korelasi antara pembentukan akrilamida dan asparagina sebagai pelopornya.

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REPLACE I certify that an Examination Committee met on 16/07/2010 to conduct the final examination of Gisia Daniali on his Mst degree of Food Science thesis entitled "Determination of acrylamide in banana-based snack and the effect of different maturity stages of banana on the formation of acrylamide in banana fritters" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The committee recommends that the student be awarded the Master of Science 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: 6 September 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 institutions.

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GISIA DANIALI

Date: 16 July 2010

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LIST OF ABBREVIATIONS

AA	Acrylamide
ACGIH	American Conference of Governmental Industrial Hygienists
Ala	Alanine
AOAC	Association of Official Analytical Chemists
ANOVA	Analysis of variance
Arg	Arginine
Asn	Asparagine
BB	Banana balls
BC	Banana chips
BCa	Banana cake
BF	Banana fritter
BMDL	Benchmark Dose Lower Limit
Br_2	Bromine
°C	Centigrade degree
CAA	Clean Air Act
CE	Capillary electrophoresis
CSTEE	Scientific Committee on Toxicity, Ecotoxicity and the Environment
EDI	Estimated daily intake
ECD	Electron capture detector
e.g.	For Example
EPA	Environmental Protection Agency
Eq	Equation

EU	European Union
EWI	Estimated weekly intake
F	Female
FAO	Food and Agricultural Organization
FDA	Food and drug administration
Frc	Fructose
FT-IR	Fourier transform infrared
g	Gram
GC	Gas chromatography
GC-AFS	Gas chromatography-atomic fluorescence spectrometry
GC-MS	Gas chromatography-mass spectrometry
Gln	Glutamine
Glc	Glucose
Gly	Glycine
h	Hour
HAP	Hazardous air pollutant
HBr	Hydrobromic acid
HCl	Hydrochloric cid
His	Histidine
HLB	Hydrophilic–lipophilic balance
HPLC-DAD	High performance liquid chromatography with diode array detection
HPLC-UV-DAD	High-performance liquid chromatography with ultraviolet diode array detection
HPLC	High-performance liquid chromatography

IARC	International Agency for Research on Cancer
Ile	Isoleucine
INIBAP	International Network for The Improvement Of Banana And Plantain
IRIS	Integrated Risk Information System
IUPAC	International Union for Pure and Applied Chemistry
JECFA	Joint Expert Committee on Food Additives
KBr	Potassium bromide
Kg	Kilogram
L	Liter
LC	Liquid chromatography
LC-MS	Liquid chromatography- mass spectrometry
LC-MS-MS	Liquid chromatography with tandem mass spectrometry
Leu	Leucine
LOAEL	lowest-observed- adverse effects level
LOD	Limit of detection
LOQ	limit of quantification
Lys	Lysine
M	Molar
MAL	Maltose
MCX	Mixed-mode cation-exchange
MeOH	Methanol
mg	Milligram
min	Minute

mL	Milliliter
mm Hg	Millimetres of mercury
MOE	Margin of exposure
MOH	Ministry of Health Malaysia
MS	Mass spectrometry
MW	Molecular Weight
NaCl	Sodium chloride
ND	Not detected
NOAEL	No observed adverse effects level
NTP	National Toxicology Program
O	Oxygen
OH^-	Hydroxide
OSHA	Occupational Safety and Health Act
PELs	Permissible exposure limits
Phe	Phenylalanine
Pro	Proline
Py-GC/MS	Pyrolysis-gas chromatography/mass spectrometry
R^2	Correlation coefficient
RQ	Reportable quantity
s	Second
SBC	Sweet banana chips
Ser	Serine
S/N	Signal/noise
SNFA	Swedish National Food Administration

SPE	Solid phase extraction
Suc	Sucrose
Thr	Threonine
Tyr	Tyrosine
USEPA	United States Environmental Protection Agency
Val	Valine
WHO	World Health Organization
µECD	Micro-electron capture detection
µg	Micro gram
µL	Micro liter

CHAPTER 1

INTRODUCTION

1.1. **Background of study**

Acrylamide (CH_2–CH–CO–NH_2) with MW 71 is a solid compound, and it is stable at room temperature (CAS No. 79–06–1). Side effects of acrylamide include drowsiness to in coordination, hallucination, and confusion. Direct contact with dissolved acrylamide irritates the skin, and acrylamide dust irritates the respiratory system (Environmental Protection Agency, 1994).

Cooking and processing of high carbohydrate foods at high temperatures have been shown to produce various kinds of cooking toxicants. The most recently detected food toxicant produced by heat processing is acrylamide (Jagerstad and Skog, 2005). Concern over acrylamide in foodstuffs arose in April 2002 when Swedish scientists reported unexpectedly high levels of this potentially carcinogenic compound in carbohydrate–rich foods heated to high temperatures (Swedish National Food Administration, 2002), since then scientists from different countries identified possible pathways for the formation of acrylamide (Mottram et al., 2002; Becalski et al., 2003; Zyzak et al., 2003). Acrylamide was found mainly in fried, deep fat fried, roasted or oven cooked foods which basely consist of carbohydrates. Only traces of acrylamide were found in boiled or braised foods, indicating that significant formation of acrylamide during processing requires temperatures of $\geq 120\ ^{o}C$ (Zyzak et al., 2003).