EFFECT OF TIME AND TEMPERATURE ON THE SIMULTANEOUS FORMATION OF HETEROCYCLIC AMINES AND POLYCYCLIC AROMATIC HYDROCARBONS USING AMINO ACID AND SUGAR MODEL SYSTEMS

By

AINAATUL ASMAA' ISHAK

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

May 2021

FSTM 2022 12

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Doctor of Philosophy

EFFECT OF TIME AND TEMPERATURE ON THE SIMULTANEOUS FORMATION OF HETEROCYCLIC AMINES AND POLYCYCLIC AROMATIC HYDROCARBONS USING AMINO ACIDAND SUGAR MODEL SYSTEMS

By

AINAATUL ASMAA' ISHAK

May 2021

Chairman : Professor Jinap Selamat, PhD
Faculty : Food Science and Technology

The aminoimidazoazaarenes types of heterocyclic amines (AIA-types of HCAs) and the 4PAH of polycyclic aromatic hydrocarbons (PAHs) are chemical compounds that form abundantly in muscle meat cooked at 150°C and above from the reaction between the amino acids and the reducing sugar. Studies on HCAs and PAHs formation are essential as both compounds have been classified as carcinogenic by the International Agency for Research on Cancer (IARC). Many studies on HCAs and PAHs focused on their formation in food samples. However, the most susceptible amino acid and sugar precursor was unable to be identified due to the complex system containing many food components. Chemical model system have the advantages of allowing researchers to study the effect of single precursor on HCAs or PAHs formation as the system contains only the precursor. From using chemical model system, previous studies have identified phenylalanine, proline, and glycine as the amino acids responsible for the formation of the AIA-types of HCAs and the 4PAH that are abundant in cooked muscle meat. Nevertheless, studies on the simultaneous formation are limited although they originated from the same precursor and most reported studies focused on HCAs and PAHs formation separately. There is also limited data on the rate formation involving kinetic studies that can be used to signify the difference between each precursor on the formation of HCAs and PAHs. Therefore, the objective of this study is to identify the most susceptible amino acid (phenylalanine, proline, and glycine) and sugar (glucose, fructose, and sucrose) precursor for the simultaneous formation of HCAs and PAHs at household cooking time and temperature using chemical model system with the adaption of kinetic study. Essentially, this study uses an amino acid model system and a sugar model system to investigate the effects of these precursors on the simultaneous formation of HCAs and PAHs. The used of phenylalanine, proline, and glycine were selected as it was identified by previous studies to from most of the HCAs and PAHs compound. Each amino acid model systems were heated at a household

cooking temperature and time ranged from 150°C to 270°C at 4 to 16 minutes. The data obtained were fitted into the first-order model equation, Arrhenius equation, and Eyring equation to determine the rate formation of HCAs and PAHs from different amino acid model systems. In sugar model system, glucose, fructose, and sucrose were chosen since they are commonly found in meat. The formation of HCAs and PAHs were identified and quantified using high performance liquid chromatography (HPLC) equipped with photo diode array (PDA) and fluorescence (FLD) detectors. Gas chromatography – mass spectrometry (GC-MS) screening on the model system containing the most susceptible amino acid and sugar was conducted to identify the possible intermediate compounds causing the formation of HCAs and PAHs and predicting the pathway formation. The findings of this study revealed that heating temperature has higher significant effect over heating time. The presence of various amino acids significantly influenced the types of HCAs and PAHs formed, whereas the presence of sugar highly influenced the amount formed. Furthermore, not all amino acid were able to form HCAs but can easily form PAHs. Phenylalanine was identified as the precursor for imidazoquinoline, imidazoquinoxaline, imidazopyridine; proline was the precursor imidazoquinoxaline, and imidazopyridine; whereas as glycine was the precursor for imidazoquinoline, and imidazoquinoxaline. Interestingly, all three amino acids were the precursor for PAHs which comprises of *cata*-condensed PAHs (benz[a]anthracene, BaA and chrysene, Chry) and *peri*-condensed PAHs (benzo[b]fluoranthen, BbF and benzo[a]pyrene, BaP). The results from the kinetic studies revealed that regardless on the types of amino acids used, the simultaneous formation of HCAs and PAHs followed the first-order model and that the reaction was an endothermic and bimolecular reaction. Based on the reaction rate (*k*) and activation energy (*Ea*) values obtained from the first-order model and the Arrhenius equation, the formations of HCAs and PAHs in each amino acid (phenylalanine, proline, and glycine) model systems were formed at a relatively different rate. All HCAs and PAHs compounds were identified in the heated system of phenylalanine. Hence, phenylalanine was identified as the most susceptible amino acid for the simultaneous formation HCAs and PAHs followed by glycine and proline. In the sugar model systems, glucose was identified as the most susceptible sugar precursor, forming high amount of HCAs and PAHs. This was then followed by fructose and sucrose. In general, the increased in the amino acid and sugar concentrations resulted in a significant increase in the simultaneous formation of HCAs and PAHs. The GC-MS screening on model system with most susceptible amino acid (phenylalanine) and sugar (glucose) precursor identified five compound namely 4-methyl quinoline, methyl-3-phenylpropanoate, 3,6-dibenzylpiperazine-2,5-dione, 3-benzyl-6-methylpiperazine-2,5-dione, and creatinine that were involved in the pathway formation of HCAs and PAHs. It can be concluded that different amino acids highly influence the types of HCAs and PAHs whereas, the reducing sugar highly influence the amount of HCAs and PAHs formed. Their simultaneously formation occurred at a relatively different rate depending on the type of amino acid presence. However, regardless on the type of amino acids, the simultaneous formation follows the first order model and the reaction was an endothermic and bimolecular reaction. Phenylalanine and glucose were identified as the most susceptible precursor for the simultaneous formation of HCAs and PAHs.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

KESAN MASA DAN SUHU TERHADAP PEMBENTUKAN SERENTAK AMINA HETEROSIKLIK DAN HIDROKARBON AROMATIK POLISIKLIK MENGGUNAKAN MODEL SISTEM ASID AMINO DAN GULA

Oleh

AINAATUL ASMAA' ISHAK

Mei 2021

Pengerusi : Profesor Jinap Selamat, PhD
Fakulti : Sains dan Teknologi Makanan

Aminaimidazoazaren daripada amina heterosiklik (AIA-jenis HCA) dan 4PAH daripada hidrokarbon aromatik polisiklik (PAH) adalah sebatian kimia yang banyak terbentuk dalam daging yang dimasak pada suhu 150°C dan ke atas hasil tindak balas antara asid amino dan gula penurun. Kajian mengenai pembentukan HCA dan PAH adalah penting kerana keuda-dua sebatian tersebut telah diklasifikasikan sebagai karsinogenik oleh *International Agency for Research on Cancer* (IARC). Kebanyakkan kajian mengenai HCA dan PAH memberi tumpuan kepada pembentukannya dalam sampel makanan. Walau bagaimanapun, asid amino dan gula penurun yang paling mudah membentuk HCA dan PAH tidak dapat dikenal pasti oleh kerana sampel makanan merupakan sistem kompleks yang mengandungi pelbagai komponen makanan. Model sistem kimia mempunyai kelebihan yang membolehkan para penyelidik mengkaji kesan satu prekursor terhadap pembentukan HCA atau PAH kerana sistem ini menggunakan campuran prekursor. Dengan menggunakan model sistem kimia, kajian terdahulu telah mengenal pasti fenilalanina, prolina, dan glisina bekebolehan untuk menghasilkan sebilangan besar sebatian HCA dan PAH. Namun begitu, kajian mengenai pembentukan serantak HCA dan PAH adalah terhad walaupun kedua-duanya terbentuk dari prekursor yang sama serta kebanyakan kajian melaporkan pembentukan HCA dan PAH secara berasingan. Selain itu, data kadar pembentukan yang melibatkan penggunaan kinetik untuk membezakan kadar pembentukan HCA dan PAH dari prekursor yang berlainan juga adalah terhad. Oleh itu, objektif kajian ini adalah untuk mengenal pasti asid amino (fenilalanina, prolina, dan glisina) dan gula (glukosa, fruktosa, dan sukrosa) yang paling mudah membentuk HCA dan PAH secara serentak pada masa dan suhu memasak menggunakan gabungan model sistem kimia dan kajian kinetik. Secara asasnya, kajian ini menggunakan model sistem asid amino dan model sistem gula untuk mengkaji kesan kedua-dua prekursor terhadap pembentukan serentak HCA dan PAH. Fenilalanina, prolina, dan glisina dipilih kerana kajian terdahulu mendapati asid

amino ini berkebolehan untuk menghasilkan sebilangan besar HCA dan PAH. Setiap model sistem asid amino dipanaskan pada suhu dan tempoh yang biasa digunakan untuk memasak iaitu antara 150°C hingga 270°C selama 4 hingga 16 minit. Data yang diperolehi digunakan dalam persamaan model peringkat-pertama, persamaan *Arrhenius*, dan *Eyring* untuk menentukan kadar pembentukan HCA dan PAH. Dalam model sistem gula, glukosa, fruktosa, dan sukrosa dipilih kerana kebiasanya yang terdapat dalam daging. Pembentukan HCA dan PAH dikenalpasti dan dikuantifikasi dengan menggunakan kromatografi cecair berprestasi tinggi (HPLC) yang dilengkapi dengan alat pengesan foto dioda (PDA) dan pendarfluor (FLD). Analisis kromatografi gas - spektrometri jisim (GC-MS) pada model sistem yang mengandungi asid amino dan gula yang paling mudah membentuk HCA dan PAH dilakukan untuk mengenal pasti sebatian perantaraan dan meramalkan laluan pembentukannya. Hasil kajian menunjukkan kesan suhu pemanasan adalah lebih signifikan berbanding dengan masa pemanasan. Kepelbagaian asid amino mempengaruhi jenis HCA dan PAH, manakala kehadiran gula mempengaruhi jumlah yang terbentuk. Tidak semua asid amino berkebolehan membentuk HCA tetapi membentuk PAH dengan mudah. Fenilalanina dikenal pasti sebagai prekursor kepada imidazokuinolina, imidazokuinoxalina, dan imidazopiridina; prolina adalah prekursor kepada imidazokuinoxalina dan imidazopiridina; manakala glisina adalah prekursor kepada imidazokuinolina dan imidazokuinoxalina. Namun begitu, ketiga-tiga asid amino itu merupakan prekursor kepada PAH yang terdiri daripada PAH *cata-condensed* dan *peri-condensed*. Hasil kajian kinetik pula menunjukkan tanpa mengira jenis asid amino yang digunakan, pembentukan serentak HCA dan PAH mengikuti model peringkat-pertama dan tindak balas yang terbentuk adalah tindak balas endoterma dan dwimolekul. Berdasarkan nilai kadar tindak balas (*k*) dan nilai tenaga pengaktifan (*Ea*) yang diperolehi dari model peringkat-pertama dan persamaan *Arrhenius*, pembentukan HCA dan PAH dalam setiap asid amino terbentuk pada kadar yang berbeza. Sistem fenilalanina berkebolehan membentuk kesemua sebatian HCA dan PAH. Oleh itu, fenilalanina dikenal pasti sebagai prekursor yang paling mudah untuk membentukan HCAs dan PAH secara serentak diikuti oleh glisina dan prolina. Dalam model sistem gula, glukosa telah dikenal pasti sebagai prekursor gula yang paling mudah membentuk HCA dan PAH dalam jumlah yang tinggi. Ini kemudian diikuti oleh fruktosa dan sukrosa. Secara amnya, peningkatan kepekatan asid amino dan gula mengakibatkan peningkatan ketara dalam pembentukan HCA dan PAH secara serentak. Analisis GC-MS pada model sistem yang mengandungi prekursor asid amino (fenilalanina) dan gula (glukosa) yang paling mudah membentuk HCA dan PAH mengenal pasti lima sebatian yang terlibat dalam laluan pembentukkannya iaitu 4-metil kuinolina, metil-3-fenilpropanoate, 3,6-dibenzilpiperazina-2,5-diona, 3-benzil-6-metilpiperazina-2,5-diona dan keratina. Dapat disimpulkan bahawa asid amino yang berlainan mempengaruhi jenis HCA dan PAH manakala gula mempengaruhi jumlah HCA dan PAH yang terbentuk. Pembentukan serentak sebatian ini berlaku pada kadar yang berbeza bergantung kepada jenis asid amino yang digunakan. Walau bagaimanapun, tanpa mengira jenis asid amino, tindak balas yang berlaku adalah tindakbalas endoterma, dwimolekul dan mengikuti model peringkat-pertama. Fenilalanina dan glukosa dikenal pasti sebagai prekursor yang paling mudah untuk pembentukan serentak HCA dan PAH.

ACKNOWLEDGEMENTS

In the name of God, the Almighty, Most Gracious, Most Merciful.

Alhamdulillah, praise to Allah for giving me the strength and hope to go through all the obstacles to complete my research work. To make this research success, I have received lots of encouragement, guidance and support from various parties.

With a deep sense of gratitude, I would like to express my sincere thanks to my supervisor Prof Dr. Jinap Selamat for the guidance, valuable advice and constant support throughout my research journey. I have gained lots of knowledge and experience from her and I am very grateful and fortunate to have her as my supervisor. I would like to thank my co-supervisor, Associate Professor Dr. Rabiha Sulaiman for her advice and guidance in conducting the modelling study and to Dr. Rashidah Sukor for the comments and constructive suggestions throughout finishing the research. Not to forget, Associate Professor Dr. Emila Abdulmalek from the Faculty of Science for her contributions and expertise in the field of chemistry.

Many thanks, to all my colleagues (Norlia, Sharina, Joshua, Farah, Aida, Atena, Shabnam, Mishelia, Raehan, Aliah, Naziruddin, Norafida, Izzati, Ezzati, Muzamer and Syima) in Chemical Food Safety Laboratory for the help, supports and motivations. No words can describe my gratitude to all of you. My deepest thanks to Puan Norliza Othman for her assistance and patience in ensuring the laboratory equipments and facilities are always in good conditions.

Finally, my greatest appreciation is dedicated to my dear parents Ishak Yussof and Khairun Nisa Abd Razi for their unwavering support, help, prayers and encouragement in ensuring the success of this study. To my beloved husband Azwan Syah Idris, thank you for the support through the ups and downs, love and care given.

Without them, this journey will not be successful and completed.

Thank You!

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

Jinap binti Selamat, PhD
Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Chairman)

Rashidah binti Sukor, PhD
Senior Lecturer
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Member)

Rabiha binti Sulaiman, **PhD**
Associate Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Member)

Emilia binti Abd Malek, PhD
Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Member)

ZALILAH MOHD SHARIFF, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 20 January 2022

Declaration by Graduate Student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any other institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and Innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature: _____ Date: _____

Name and Matric No.: Ainaatul Asmaa' binti Ishak

Declaration by Members of Supervisory Committee

This is to confirm that:

- The research conducted, and the writing of this thesis was under our supervision;
- Supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

Signature: _____
Name of Chairman of Supervisory Committee: Professor Dr. Jinap Selamat

Signature: _____
Name of Member of Supervisory Committee: Associate Professor Dr. Rabiha Sulaiman

Signature: _____
Name of Member of Supervisory Committee: Associate Professor Dr. Emila Abd Malek

Signature: _____
Name of Member of Supervisory Committee: Dr. Rashidah Sukor

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

AIA	Aminoimidazoazaarenes
BaA	Benz[a]anthracene
BaP	Beno[a]pyrene
BbF	Benzo[b]fluoranthen
Chry	Chrysene
DEG	Diethylene glycol
DNA	Deoxyribonucleic acid
Ea	Activation energy
EU	European Union
FLD	Fluorescence detector
GC-MS	Gas chromatography-mass spectrometry
HCAs	Heterocyclic amines
HPLC	High performance liquid chromatography
IARC	International Agency for Research on Cancer
IQ	2-Amino-3-methylimidazo[4,5-*f*]quinoline
IQx	2-Amino-3-methylimidazo[4,5-*f*]quinoxaline
k	Reaction rate
LOD	Limit of detection
LOQ	Limit of quantification
MeIQ	2-Amino-3,4-dimethylimidazo[4,5-*f*]quinoline
MeIQx	2-Amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline
PAHs	Polycyclic aromatic hydrocarbons
PDA	Photodiode array

PhIP	2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine
ppb	Parts per billion
ppm	Parts per million
°C	Degree celsius
ng/g	Nanogram/gram
g/mol	Gram/mole
µg/g	Microgram/gram
µL	Microliter
$\Delta H^{\ddagger}$	Activation enthalpy
$\Delta S^{\ddagger}$	Activation entropy

CHAPTER 1

INTRODUCTION

1.1 Study background

Heterocyclic amines (HCAs) and polycyclic aromatic hydrocarbons (PAHs) are compounds formed in cooked muscle meat as a result of thermal processing (Singh et al., 2016; Puangsombat et al., 2012; El Badry, 2010; Iwasaki et al., 2010; Jahurul et al., 2010). The presence of HCAs and PAHs in cooked muscle meat are of major concern, as both compounds have been classified as carcinogenic to humans (Group 1), probably carcinogenic to humans (Group 2A), and possibly carcinogenic to humans (Group 2B) by the International Agency for Research on Cancer (IARC). Among the groups of HCAs and PAHs, the aminoimidazoazaarenes (AIA) types of HCAs and the four PAHs were abundantly found in cooked muscle meat. The four PAHs are known as benzo[a]anthracene (BaA), chrysene (Chry), benzo[b]fluoranthene (BbF), and benzo[a]pyrene (BaP). Whereas, the AIA can be classified into three groups of:

i- Imidazoqunoline: 2-amino-3-methylimidazo[4.5-*f*]quinoline (IQ) and 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline (MeIQ)
ii- Imidazoquinoxaline: 2-amino-3-methylimidazo[4,5-*f*]quinoxaline (IQx) and 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx)
iii- Imidazopyridine: 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) (Cheng et al., 2006; Schut and Snyderwine, 1999)

The presences of HCAs and PAHs in cooked muscle meat have been documented in numerous studies. Examples of cooked meat products with high HCAs and PAHs content are grilled beef and chicken (15 – 1185 ng/g HCAs; 48 – 342 ng/g PAHs), fried chicken (23 ng/g HCAs; 10 ng/g PAHs), chicken and beef satay (14 – 39 ng/g HCAs; 14 – 132 ng/g PAHs), and smoked fish (8 ng/g HCAs; 255 – 263 ng/g PAHs) (Onwukeme et al., 2015; Puangsombat et al., 2012; Alomirah et al., 2011; Farhadian et al., 2010; Iwasaki et al., 2010; Jahurul et al., 2010). Previous studies suggested that free amino acids and reducing sugars are the precursors that trigger the formation of HCAs and PAHs via Maillard reaction by heating chemical model systems at 125 – 230°C and 600 – 900°C, respectively (Gibis, 2016; Dennis et al., 2015; Britt et al., 2004; Murkovic, 2004a, 2004b; Sharma et al., 2004). Despite the fact that meat contains a small amount of reducing sugars and mixtures of amino acids at varying concentrations, previous studies discovered that among the amino acids, phenylalanine and glycine formed the majority of HCAs compounds, while phenylalanine and proline formed the majority of PAHs compounds (Britt et al., 2004; Murkovic, 2004a, 2004b; Wang et al., 2004a; Sharma et al., 2003; Zochling and Murkovic, 2002). The presence of these amino acids in muscle meat ranged from 1.2 – 4.5 µmol/g for phenylalanine, 1.9 – 10 µmol/g for

proline, and 9.0 – 21.1 μmol/g for glycine (Borgen et al., 2001; Skog et al., 2000).

Bordas et al. (2004) discovered that, the addition of phenylalanine, glycine and alanine into a meat extract model system heated at 175°C for 2 hours, enhanced the formation of imidazoquinoxaline type-HCAs namely IQx (43 ng/g) and MeIQx (478 ng/g) which was not identified earlier in the meat extract model system. The addition of the amino acids also increased the formation of imidazopyridine type-HCAs namely PhIP from 16.7 ng/g to 83 ng/g. Another study by Borgen et al. (2001) also discovered high formation of imidazoquinoxaline type-HCAs (MeIQx and DiMeIQx) in pork chop model system and high formation of imidazopyridine type-HCA (PhIP) in chicken breast model system. Based on the amino acid profiles, pork chop contains 21.1 μmol/gdm of glycine and 3.6 μmol/gdm of phenylalanine while chicken breast contains 12.8 μmol/gdm of glycine and 4.5 μmol/gdm of phenylalanine. The formation of imidazopyridine type-HCAs (PhIP) using chemical model system containing mixtures of phenylalanine, creatinine, and glucose in a ratio of 2:2:1 heated at 128 – 200 °C for 10 – 60 minutes have been reported by Moon and Shin (2013a, 2003b), and Zochling and Murkovic (2002). Furthermore, Grivas et al. (1986), Kato et al. (1996) and Jokic et al. (2001) have shown that heated mixtures of glycine, creatinine, and glucose formed the imidazoquinoline (IQ and MeIQ) and the imidazoquinoxaline type-HCAs (IQx and MeIQx).

Majority of studies on PAHs formation used amino acid pyrolysis at extremely high temperature ranging from 600 – 900°C. Not much study reported on model system based on meat, mixture of amino acids, and mixture of amino acids with sugar. Among the amino acids that were identified to generate PAHs were leucine and glutamic acids discovered by Masuda et al. (1967), phenylalanine, proline, and serine by Nie et al. (2018), and asparagine by Sharma et al. (2009). However, studies by Nie et al. (2018), Britt et al. (2004), and Wang et al. (2004) discovered that the pyrolysis of phenylalanine at 600 °C and above, enhanced the formation of up to 12 PAH compounds including the 4PAH (BaA, Chry, BbF, and BaP).

Reducing sugar was also involved in the Maillard reaction for HCAs and PAHs formation. Nor Hasyimah et al. (2018) and Hasnol et al. (2014) reported that marinating meat with herbs and spices increased glucose levels from 0.2 g/100g to 0.9 g/100g, and marinating chicken with honey increased glucose levels from 1.60 g/100g to 3.68 g/100g respectively. The increase in glucose levels had a significant effect on the formation of HCAs compound. The use of chemical model system containing mixtures of amino acid with different reducing sugars such as glucose, fructose, galactose, and lactose resulted in the increased of HCAs and PAHs formations (Nie et al., 2018; Dennis et al., 2015; Britt et al., 2004). In addition, mixtures of glycine and creatinine with non-reducing sugar such as sucrose also showed increased in the mutagenic activity from Salmonella strain TA989 test, which later corresponded to imidazoquinoxaline type-HCAs (Skog and Jägerstad, 1990).

The formations of HCAs and PAHs were also influenced by other factors such as heating time, heating temperature, water activity and pH. The effect of heating time and heating temperature on the formation of HCAs and PAHs referred to as the cooking method. A model system was recently constructed to determine the time and temperature dependence on HCAs and PAHs formation. In general, the formation of HCAs and PAHs increased with the increase in heating time and temperature (Gibis, 2016; Alaejos and Afonso, 2011; Cheng et al., 2006). Water serves as transport medium for water soluble precursors to be transported to the surface of the product, exposing to higher temperature and increasing the formation of HCAs and PAHs (Gibis, 2016; Cheng et al., 2006). It is also known that pH value of the medium influence the Maillard reaction (Linghu et al., 2020). At pH 3 – 7, the concentration of Amadori compound increased, resulting in high HCAs formation (Linghu et al., 2020; Cremer and Eichner, 2000; Puangsombat et al., 2012).

Previous studies have also incorporated kinetics study to predict the reaction rate of each individual HCAs compounds (Moon and Shin, 2013a; Arvidsson et al., 1997). The kinetic study was applied in a model systems containing mixtures of several amino acids, mixtures of phenylalanine with creatinine and glucose, meat juice, and in meat emulsion (Ahn and Grün, 2005; Hwang and Ngadi, 2002; Arvidsson et al., 1999). The use of kinetic parameters has allowed researchers to determine and differentiate the reaction rate (speed of reaction) of each individual HCAs compounds in the system.

1.2 Problem statement

The simultaneous formation of HCAs and PAHs is unavoidable. Nonetheless, the formation's level is controllable. To control the levels of formation, it is necessary to first understand the major factor that influences the formation of HCAs and PAHs. The major factor that caused the formation of HCAs and PAHs is the presences of amino acids and reducing sugar in meat that is believed to be a precursor for HCAs and PAHs. The formation was triggered via Maillard reaction between the amino acids and the reducing sugars in the presences of heating temperature and heating time. Based on previous studies, phenylalanine, proline, glycine, and reducing sugar have been identified as the precursors that formed the majority of HCAs and PAHs compounds.

Several studies have documented the use of chemical model system to study the formation of HCAs and PAHs. However, limited data was available on the simultaneous formation even though these compounds were derived from the same precursors. Up to date, the incorporation on kinetic study in chemical model system for studying the simultaneous formation of HCAs and PAHs has not been reported. The use of kinetic studies is extremely beneficial for determining the reaction rate of each individual HCAs and PAHs compound as a function of time and temperature from various precursors. Furthermore, the majority of reported studies on PAHs formation focused on PAHs occurrence in

the environment via amino acids pyrolysis at extremely high temperature of more than 600°C, which is not a common practise in household cooking methods.

Therefore, the aim of this research is to identify the most susceptible amino acid and sugar precursors among phenylalanine, proline, glycine, reducing and non-reducing sugars using an amino acid model system and a sugar model system heated at commonly used household cooking times and temperatures, as well as to predict the pathway formation from the most susceptible amino acid and sugar precursor by identifying the intermediate compounds.

1.3 Significance of study

The use of chemical model systems (amino acid model system and sugar model system) in this study that only include the precursors (amino acids, sugar, and creatinine) allows researchers to modify the precursor content in order to investigate the effect of single precursor on the simultaneous formation of HCAs and PAHs. This will be a difficult task if food model system was used since other food components in the food may interfere with HCAs and PAHs formation. The amino acid precursor namely phenylalanine, proline, and glycine were chosen since they have previously been shown to be capable of forming majority of the HCAs and PAHs (Nie et al., 2018; Wang et al., 2004a; Jägerstad et al., 1991).

The adaptation of kinetic studies in each amino acid model systems can differentiate the rate formation of each individual HCAs and PAHs compounds. Heating the system at common household cooking time and temperature may provide insight information on how these compounds are formed during cooking. Furthermore, because sugar is also a component of muscle meat composition, the data obtained from sugar model systems can be used to identify the significant effect of reducing sugar and non-reducing sugar on the simultaneous formation of HCAs and PAHs. Identifying the intermediate compounds and predicting the pathway formation of HCAs and PAHs can provide better understanding of how these compounds are form.

It is important to understand how HCAs and PAHs are formed as these compounds can form DNA adduct in the human body exposing to the risk of cancer. The data obtained from this study can contribute to the existing database of HCAs or PAHs formation using chemical model system. Furthermore, the findings can also provide awareness for consumer into the development of methods and precautions steps for the household cooking method or commercial processing to reduce or control the formation of these carcinogenic and mutagenic compounds.

1.4 Objectives of the study

This research will cover three objectives as follows:

1. To determine the effect of time and temperature on the simultaneous formation of HCAs and PAHs in three different amino acid model systems and their formation kinetics.

2. To determine the most susceptible amino acids precursor among phenylalanine, proline, and glycine for the simultaneous formation HCAs and PAHs.

3. To evaluate the role of reducing sugar and non-reducing sugar in the simultaneous formation of HCAs and PAHs and predicting the pathway formation through intermediate compounds.

1.5 Hypothesis of the study

1. The simultaneous formation of HCAs and PAHs will increase as the heating time and temperature increase in phenylalanine, proline, and glycine model systems.

2. Among the amino acids, phenylalanine will be the most susceptible amino acid precursor for HCAs and PAHs formation compare to proline and glycine.

3. Both reducing sugar and non-reducing sugar influence the concentrations of HCAs and PAHs. HCAs and PAHs forms through different pathways involving different intermediate compounds.

1.6 Research flow

Figure 1.1 and Figure 1.2 illustrate the amino acid model system and sugar model system, which will be use in the research.

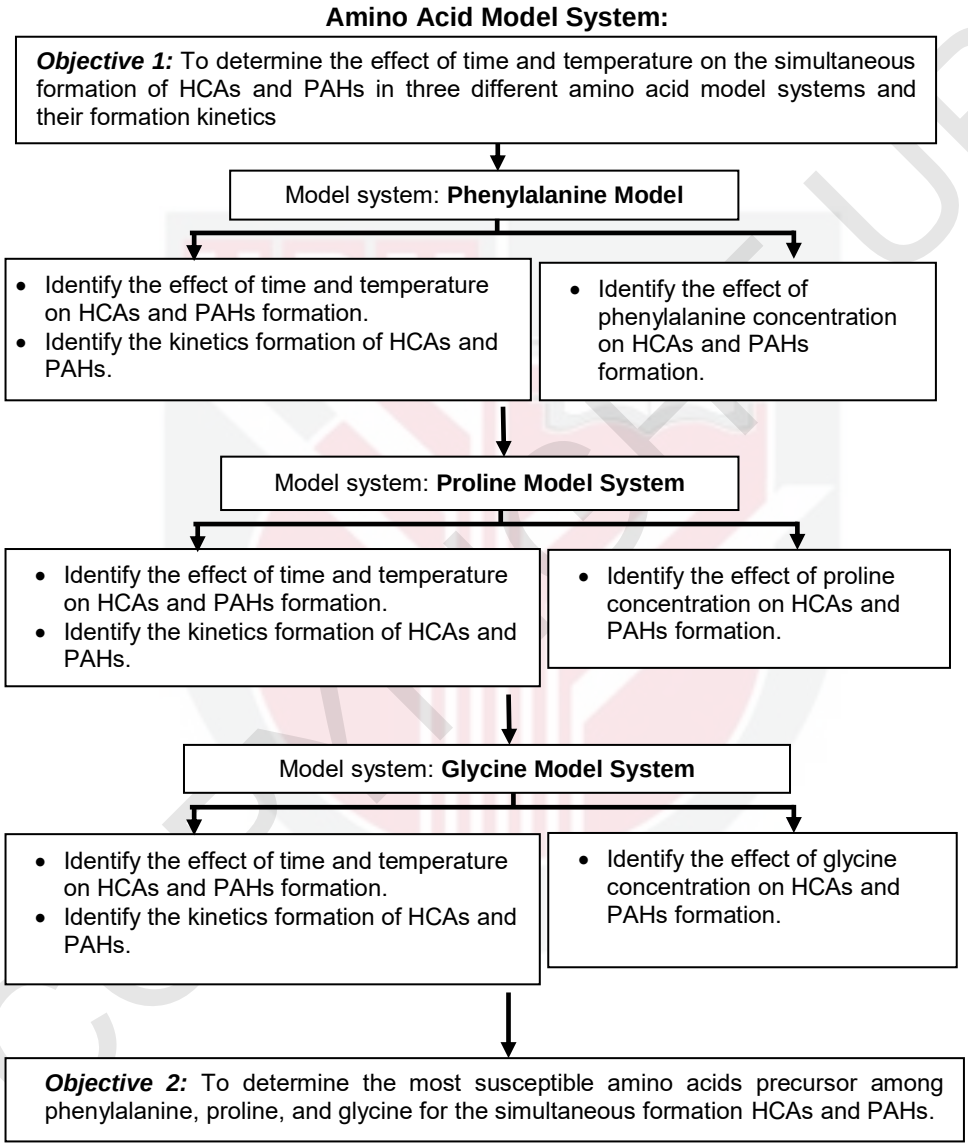

Figure 1.1 : Flow chart of amino acid model system

Sugar Model System:

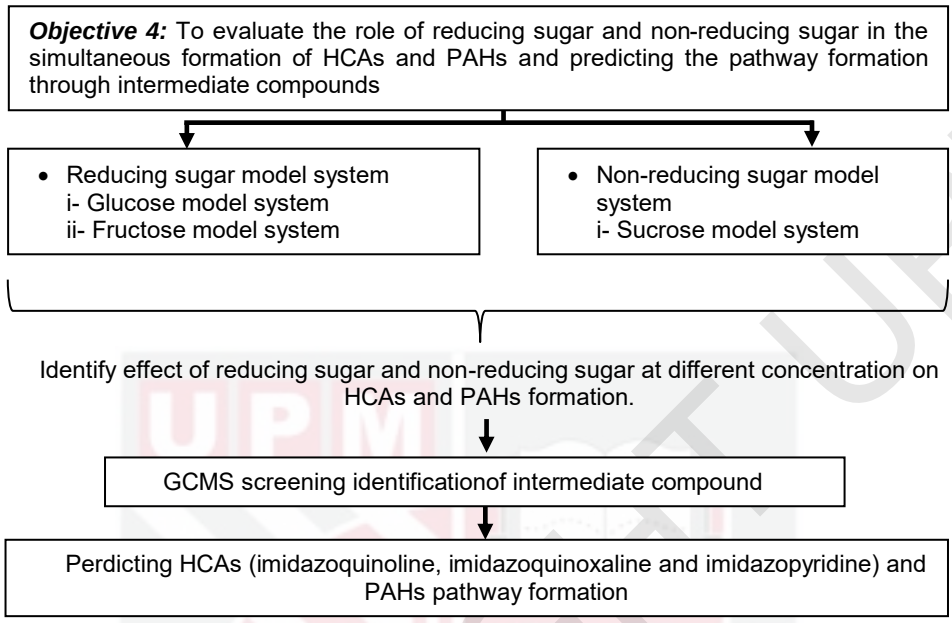

Figure 1.2 : Flow chart of sugar model system

REFERENCES

Aaslyng, M. D., Duedahl-Olesen, L., Jensen, K., and Meinert, L. (2013). Content of heterocyclic amines and polycyclic aromatic hydrocarbons in pork, beef and chicken barbecued at home by Danish consumers. *Meat Science*, *93*, 85–91.

Abdel-Shafy, H. I., and Mansour, M. S. M. (2016). A review on polycyclic aromatic hydrocarbons: Source, environmental impact, effect on human health and remediation. *Egyptian Journal of Petroleum*, *25*, 107–123.

Adeyeye, S. A. O. (2018). Heterocyclic amines and polycyclic aromatic hydrocarbons in cooked meat products: A review. *Polycyclic Aromatic Compounds*, 1–11.

Ahn, J., and Grün, I. U. (2005). Heterocyclic amines: 1. Kinetics of formation of polar and nonpolar heterocyclic amines as a function of time and temperature. *Journal of Food Science*, *70*(2), C173–C179.

Alaejos, M. S., Pino, V., and Afonso, A. M. (2008). Metabolism and toxicology of heterocyclic aromatic amines when consumed in diet: Influence of the genetic susceptibility to develop human cancer. *Food Research International*, *41*, 327–340.

Alomirah, H., Al-zenki, S., Al-hooti, S., Zaghloul, S., Sawaya, W., Ahmed, N., and Kannan, K. (2011). Concentrations and dietary exposure to polycyclic aromatic hydrocarbons (PAHs) from grilled and smoked foods. *Food Control*, *22*, 2028–2035.

Arvidsson, P., Van Boekel, M. A. J. S., Skog, K., and Jägerstad, M. (1997). Kinetics of formation of polar heterocyclic amines in a meat model system. *Journal of Food Science*, *62*(5), 911–916.

Arvidsson, P., Van Boekel, M. A. J. S., Skog, K., Solyakov, A., and Jägerstad, M. (1999). Formation of heterocyclic amines in a meat juice model system. *Journal of Food Science*, *64*(2), 216–221.

Barzegar, F., Kamankesh, M., and Mohammadi, A. (2019). Heterocyclic aromatic amines in cooked food: A review on formation, health risk-toxicology and their analytical techniques. *Food Chemistry*, *280*, 240–254.

Bordas, M., Moyano, E., Puignou, L., and Galceran, M. T. (2004). Formation and stability of heterocyclic amines in a meat flavour model system: Effect of temperature, time and precursors. *Journal of Chromatography B*, *802*, 11–17.

Borgen, E., Solyakov, A., and Skog, K. (2001). Effects of precursor composition and water on the formation of heterocyclic amines in meat model systems. *Food Chemistry*, *74*, 11–19.

Britt, P. F., Buchanan, A. C., Owens Jr, C. V, and Skeen, J. T. (2004). Does glucose enhance the formation of nitrogen containing polycyclic aromatic compounds and polycyclic aromatic hydrocarbons in the pyrolysis of proline. *Fuel*, *83*, 1417–1432.

Brown, A. M. (2001). A step-by-step guide to non-linear regression analysis of experimental data using a microsoft excel spreadsheet. *Computer Methods and Programs in Biomedicine*, *65*, 191–200.

Busquets, R., Bordas, M., Toribio, F., Puignou, L., and Galceran, M. T. (2004). Occurrence of heterocyclic amines in several home-cooked meat dishes of the Spanish diet. *Journal of Chromatography B*, *802*, 79–86.

Chang, R. (2005). Enzyme kinetics. In R. Chang (Ed.), *Physical Chemistry for the Biosciences* (pp. 338–342). California: University Science Books.

Chang, R. (2007). Thermochemistry and Chemical Kinetics. In R. Chang (Ed.) *Chemistry* (pp. 224–558). New York: McGraw Hill.

Chen, B. H., and Meng, C. N. (1999). Formation of heterocyclic amines in a model system during heating. *Journal of Food Protection*, *62*(12), 1445–1450.

Chen, Y. C., and Chen, B. H. (2001). Stability of polycyclic aromatic hydrocarbons during heating. *Journal of Food and Drug Analysis, 9*(1), 33–39.

Cheng, K. W., Chen, F., and Wang, M. (2006). Heterocyclic amines: Chemistry and health. *Molecular Nutrition and Food Research*, *50*, 1150–1170.

Cheng, K. W., Wong, C. C., Chao, J., Lo, C., Chen, F., Chu, I. K., Che, C. M., Ho, C, T and Wang, M. (2009). Inhibition of mutagenic PhIP formation by epigallocatechin gallate via scavenging of phenylacetaldehyde. *Molecular Nutrition and Food Research*, *53*, 716–725.

Chiavari, G., and Galletti, G. C. (1992). Pyrolysis-gas chromatography/mass spectrometry of amino acids. *Journal of Analytical and Applied Pyrolysis*, *24*, 123–137.

Chiu, C. P., and Chen, B. H. (2000). Stability of heterocyclic amines during heating. *Food Chemistry*, *68*, 267–272.

Dennis, C., Karim, F., and Smith, J. S. (2015). Evaluation of maillard reaction variables and their effect on heterocyclic amine formation in chemical model systems. *Journal of Food Science*, *80*(2), 472–478.

Dong, A., Lee, J., and Shin, H. S. (2011). Formation of amino-imidazo-azaarenes and carbolines in fried beef patties and chicken breasts under different cooking conditions in Korea. *Food Science and Biotechnology*, *20*(3), 735–741.

El Badry, N. (2010). Effect of household cooking methods and some food additives on polycyclic aromatic hydrocarbons (PAHs) formation in chicken meat. *World Applied Sciences Journal*, *9*, 963–974.

United States of Environmental Protection Agency (EPA). (1998). EPA-454/R-98-014 Locating and estimationg air emissions form sources of polycyclic organic matter. Retrieved 11 Februari 2019 from http://www.epa.gov/ttn/chief

Ess, D. H., Liu, S., and Proft, F. D. (2010). Density functional steric analysis of linear and branched alkanes. *Journal of Physical Chemistry A*, *114*, 12952–12957.

European Union. (2011a). Commission Regulation (EU) No 835/2011 of August 2011 amending Regulation (EU) No 1881/2006 as regards maximum levels for polycyclic aromatic hydrocarbons in foodstuffs. *Official Journal of the European Union*, *L214:5*. Retrieved 16 September 2019 from https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:215:0004:0008:EN:PDF

European Union. (2011b). Commission Regulation (EU) No 836/2011 of 19 August 2011 amending Regulataion (EC) No 333/2007 laying down the methods of sampling and analysis for the official control of the levels lead, cadnium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in food. *Official Journal of the European Union*, *L215:9*. Retrieved 16 September 2019 from https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:215:0009:0016:EN:PDF

Farhadian, A., Jinap, S., Abas, F., and Sakar, Z. I. (2010). Determination of polycyclic aromatic hydrocarbons in grilled meat. *Food Control*, *21*, 606–610.

Farhadian, A., Jinap, S., Faridah, A., and Zaidul, I. S. M. (2012). Effects of marinating on the formation of polycyclic aromatic hydrocarbons (benzo[a]pyrene, benzo[b]fluoranthene and fluoranthene) in grilled beef meat. *Food Control*, *28*, 420–425.

Farhadian, A., Jinap, S., Hanifah, H. N., and Zaidul, I. S. (2011). Effects of meat preheating and wrapping on the levels of polycyclic aromatic hydrocarbons in charcoal-grilled meat. *Food Chemistry*, *124*, 141–146.

Gibis, M. (2016). Heterocyclic aromatic amines in cooked meat products: Causes, formation, occurrence, and risk assessment. *Comprehensive Reviews in Food Science and Food Safety*, *15*, 269–302.

Grivas, S., Nyhammar, T., and Olsson, K. (1986). Isolation and identification of the food mutagens IQ and MeIQx from a heated model system of creatinine, glycine and fructose. *Food Chemistry*, *20*, 127–136.

Grivas, S., Nyhammar, T., Olsson, K., and Jägerstad, M. (1985). Formation of a new mutagenic DiMeIQx compound in a model system by heating creatinine, alanine and fructose. *Mutation Research*, *151*, 177–183.

Gross, G. A., and Grüter, A. (1992). Quantitation of mutagmic/carcinogenic heterocyclic aromatic amines in food products. *Journal of Chromatography*, *592*, 271–278.

Guillén, M. D., and Sopelana, P. (2003). Polycyclic Aromatic Hydrocarbons in Diverse Foods. In J. P. F. D'Mello (Ed.), *Food Safety: Contaminants and Toxins* (pp. 175–198). Edinburgh: CABI Publising.

Hamidi, E. N., Hajeb, P., Jinap, S., and Razis, A. F. A. (2016). Polycyclic aromatic hydrocarbons (PAHs) and their bioaccessibility in meat: A tool for assessing human cancer risk. *Asian Pacific Journal of Cancer Prevention*, *17*(1), 15–23.

Hasnol, N. D. S., Jinap, S., and Sanny, M. (2014). Effect of different types of sugars in a marinating formulation on the formation of heterocyclic amines in grilled chicken. *Food Chemistry*, *145*, 514–521.

House, J. E. (2007). Kinetics of More Complex Systems. In J. E. House (Ed), *Principle of Chemical Kinetics* (pp. 37–75). London: Accedemic Press.

Hwang, D. K., and Ngadi, M. (2002). Kinetics of heterocyclic amines formation in meat emulsion at different fat contents. *Food Science and Technology*, *35*, 600–606.

IARC. (1993). Some Naturally Occurring Substances: Food Items and Constituents, Heterocyclic Aromatic Amines and Mycotoxins. In *IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Human* (Vol. 56, pp. 245–395). Lyon, France: International Agency for Research on Cancer.

Iwasaki, M., Kataoka, H., Ishihara, J., Takachi, R., Hamada, G. S., Sharma, S., Marchand, L. L and Tsugane, S. (2010). Heterocyclic amines content of meat and fish cooked by Brazilian methods. *Journal of Food Composition and Analysis*, *23*, 61–69.

Jägerstad, M., Skog, K., Grivas, S., and Olsson, K. (1991). Formation of heterocyclic amines using model systems. *Mutation Research*, *259*, 219–233.

Jahurul, M. H. A., Jinap, S., Ang, S. J., Abdul-Hamid, A., Hajeb, P., Lioey, H. N., and Zaidul, I. S. M. (2010). Dietary exposure to heterocyclic amines in high-temperature cooked meat and fish in Malaysia. *Food Additives and Contaminants - Part A*, *27*(8), 1060–1071.

Jamin, E. E., Riu, A., Douki, T., Debrauwer, L., Cravedi, J. P., Zalko, D., and Audebert, M. (2013). Combined genotoxic effects of a polycyclic aromatic hydrocarbon (B(a)P) and an heterocyclic amine (PhIP) in relation to colorectal carcinogenesis. *PLoS ONE*, *8*(3), 1–11.

Janoszka, B., Warzecha, L., Blaszcyk, U., and Bodzek, D. (2004). Organic compounds formed in thermally treated high-protein food part I : Polycyclic aromatic hydrocarbons. *Acta Chromatographic*, 14, 115–128.

Jinap, S., Mohd-Mokhtar, M. S., Farhadian, A., Hasnol, N. D. S., Jaafar, S. N., and Hajeb, P. (2013). Effects of varying degrees of doneness on the formation of heterocyclic aromatic amines in chicken and beef satay. *Meat Science*, *94*, 202–207.

Johansson, M. A. E., Laurent, B. F., Gross, G. A., Olsson, K., and Jägerstad, M. (1995). Influence of amino acids on the formation of mutagenic/carcinogenic heterocyclic amines in a model system. *Carcinogenesis*, *16*(10), 2553–2560.

John, E. M., Stern, M. C., Sinha, R., and Jocelyn, K. (2011). Meat consumption, cooking practices, meat mutagens, and risk of prostate cancer. *Nutrition and Cancer*, *63*(4), 525–537.

Jokic, A., Zimpel, Z., Huang, P. M., and Mezey, P. G. (2001). Molecular shape analysis of a maillard reaction intermediate. *SAR and QSAR in Environmental Research*, *12*, 297–307.

Kato, T., Harashima, T., Moriya, N., Kikugawa, K., and Hiramoto, K. (1996). Formation of the mutagenic/carcinogenic imidazoquinoxaline-type heterocyclic amines through the unstable free radical Maillard intermediates and its inhibition by phenolic antioxidants. *Carcinogenesis*, *17*(11), 2469–2476.

Keating, G. A., and Bogen, K. T. (2004). Estimates of heterocyclic amine intake in the US population. *Journal of Chromatography B*, *802*, 127–133.

Kim, S., and Lee, K. G. (2010). Effects of cooking variables on formation of heterocyclic amines (HCA) in roasted pork and mackerel. *Journal of Toxicology and Environmental Health*, *73*(21–22), 1599–1609.

Kizil, M., Oz, F., and Besler, H. T. (2011). A review on the formation of carcinogenic/mutagenic heterocyclic aromatic amines. *Journal of Food Processing and Technology*, *2*(5), 2–5.

Knize, M. G., and Felton, J. S. (2005). Formation and human risk of carcinogenic heterocyclic amines formed from natural precursors in meat. *Nutrition Reviewes*, *63*(5), 158–165.

Kondjoyan, A., Chevolleau, S., Grève, E., Gatellier, P., Santé-lhoutellier, V., Bruel, S., Touzet, C., Portanguen, S, and Debrauwer, L. (2010a). Formation of heterocyclic amines in slices of Longissimus thoracis beef muscle subjected to jets of superheated steam. *Food Chemistry*, *119*(1), 19–26.

Kondjoyan, A., Chevolleau, S., Grève, E., Gatellier, P., Santé-lhoutellier, V., Bruel, S., Touzet, C., Portanguen, S, and Debrauwer, L. (2010b). Modelling the formation of heterocyclic amines in slices of longissimus thoracis and semimembranosus beef muscles subjected to jets of hot air. *Food Chemistry*, *123*(3), 659–668.

Linghu, Z., Karim, F., Taghvaei, M., Albashabsheh, Z., Houser, T. A., and Smith, J. S. (2020). Amino acids effects on heterocyclic amines formation and physicochemical properties in pan-fried beef patties. *Journal of Food Science*, *85*(4), 1361–1370.

Linghu, Z., Karim, F., and Smith, J. S. (2017). Amino acids inhibitory effects and mechanism on 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) formation in the maillard reaction model systems. *Journal of Food Science*, *82*(12), 3037–3045.

Lund, M. N., and Ray, C. A. (2017). Control of Maillard reactions in foods: strategies and chemical mechanisms. *Journal of Agricultural and Food Chemistry*, *65*, 4537–4552.

Masuda, Y., Mori, K., and Kuratsune, M. (1967). Polycyclic aromatic hydrocarbons formed by pyrolysis of carbohydrates, amino acids, and fatty acids. *Japanese Journal of Cancer Research-Gann*, *58*, 69–74.

Moon, S. E., and Shin, H. S. (2013a). Formation of genotoxic 2-amino-1-methyl-6-phenylimidazo [4,5-b] pyridine (PhIP) and its kinetics in a model system. *Food Science and Biotechnology*, *22*, 137–145.

Moon, S. E., and Shin, H. S. (2013b). Inhibition of mutagenic 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) formation using various food ingredients in a model systems. *Food Science and Biotechnology*, *22*(2), 323–329.

Mumtaz, M. M., George, J. D., Gold, K. W., Cibulas, W., and Derosa, C. T. (1996). ATSDR evaluation of health effects of chemicals. Iv. Polycyclic aromatic hydrocarbons (PAHs): Understanding a comples problem. *Toxicology and Industrial Health*, *12*(6), 742–995.

Muñoz, B., and Albores, A. (2011). DNA Damage Caused by Polycyclic Aromatic Hydrocarbons: Mechanisms and Markers. In C. Chen (Ed.), *Selected Topics in DNA Repair* (pp. 125–144). Rijeka: InTech.

Murkovic, M. (2004a). Chemistry, formation and occurrence of genotoxic heterocyclic aromatic amines in fried products. *European Journal of Lipid Science and Technology*, *106*, 777–785.

Murkovic, M. (2004b). Formation of heterocyclic aromatic amines in model systems. *Journal of Chromatography B*, *802*, 3–10.

Negishi, C., Wakabayashi, K., Tsuda, M., Sato, S., Sugimura, T., Saito, H., Maeda, M and Jägerstad, M. (1984). Formation of 2-amino-3,7,8-trimethylimidazo[4,5-f]quinoxaline, a new mutagen, by heating a mixture of creatinine, glucose and glycine. *Mutation Research*, *140*, 55–59.

Nie, W., Cai, K. Z., Li, Y. Z., Zhang, S., Wang, Y., Guo, J., Chen, C. G and Xu, B. C. (2018). Small molecular weight aldose (D-glucose) and basic amino acids (L-lysine, L-arginine) increase the occurrence of PAHs in grilled pork sausages. *Molecules*, *23*, 1–12.

National Toxicology Program. (2002). Heterocyclic Amines (Selected). In *Report on Carcinogens, Fourteenth Edition*. Retrieved 17 February 2015 from http://ntp.niehs.nih.gov/ntp/roc/content/profiles/heterocyclicamines.pdf

Nor Hasyimah, A. K., Jinap, S., and Sanny, M. (2018). Simultaneous formation of polycyclic aromatic hydrocarbons (PAHs) and heterocyclic aromatic amines (HCAs) in gas-grilled beef satay at different temperatures. *Food Additives and Contaminants: Part A*, *35*(5), 848–869.

Onwukeme, V. I., Obijiofor, O. C., Asomugha, R. N., and Okafor, F. A. (2015). Impact of cooking methods on the levels of polycyclic aromatic hydrocarbons (PAHs) in chicken meat. *Journal of Environmental Science, Toxicology and Food Technology*, *9*(4), 21–27.

Overvik, E., Kleman, M., Berg, I., and Gustafsson, J. A. (1989). Influence of creatine, amino acids and water on the formation of the mutagenic heterocyclic amines found in cooked meat. *Carcinogenesis*, *10*(12), 2293–2301.

Oz, F., Kaban, G., and Kaya, M. (2010). Effects of cooking techniques and levels on the formation of heterocyclic aromatic amines in chicken and fish. *Journal of Animal and Veterinary Advances*, *9*(8), 1259–1264.

Pais, P., Salmon, C. P., Knize, M. G., and Felton, J. S. (1999). Formation of mutagenic/carcinogenic heterocyclic amines in dry-heated model systems, meats, and meat drippings. *Journal of Agriculture and Food Chemistry*, *47*, 1098–1108.

Parker, J. K. (2015). Thermal generation or aroma. In J. K. Parker, J. S. Elmore, and L. Methven (Eds.), *Flavour Development, Analysis and Perception in Food and Beverages* (pp. 151–185). Kidlington: Elsevier Ltd.

Pearson, A. M., Chen, C., Gray, J. I., and Aust, S. D. (1992). Mechanism(s) involved in meat mutagen formation and inhibition. *Free Radical Biology and Medicine*, *13*, 161–167.

Persson, E., Sjoholm, I., and Skog, K. (2003). Effect of high water-holding capacity on theformation of heterocyclic amines in fried beefburgers. *Journal of Agricultural and Food Chemistry*, *51*(15), 4472–4477.

Perelló, G., Martí-cid, R., Castell, V., Llobet, J. M., and Domingo, J. L. (2009). Concentrations of polybrominated diphenyl ethers, hexachlorobenzene and polycyclic aromatic hydrocarbons in various foodstuffs before and after cooking. *Food and Chemical Toxicology*, *47*, 709–715.

Philip, E. K., and Odell, G. V. (1970). Factors affecting the formation of pyrazine compounds in sugar-amine reactions. *Journal of Agricultural and Food Chemistry*, *18*(5), 895–898.

Poater, J., Visser, R., Sola, M., and Bickelhaupt, F. M. (2007). Polycyclic benzenoids: Why kinked is more stable than straight. *Journal of Organic Chemistry*, *72*, 1134–1142.

Puangsombat, K., Gadgil, P., Houser, T. A., Hunt, M. C., and Smith, J. S. (2012). Occurrence of heterocyclic amines in cooked meat products. *Meat Science*, *90*, 739–746.

Raj, G. (2010). First order reactions. In G. Raj (Ed), *Chemical Kinetics* (pp. 25–36). Meerut: Krishna Prakashan Media.

Ramesh, A., Walker, S. A., Hood, D. B., Guillén, M. D., Schneider, K., and Weyand, E. H. (2004). Bioavailability and risk assessment of orally ingested polycyclic aromatic hydrocarbons. *International Journal of Toxicology, 23*, 301–333.

Reizer, E., Csizmadia, I. G., Palotas, A., Viskolcz, B., and Fiser, B. (2019). Formation mechanism of benzo(a)pyrene: One of the most carcinogenic polycyclic aromatic hydrocarbons (PAH). *Molecules*, *24*(1040), 1–12.

Rengarajan, T., Rajendran, P., Nandakumar, N., Lokeshkumar, B., Rajendran, P., and Nishigaki, I. (2015). Exposure to polycyclic aromatic hydrocarbons with special focus on cancer. *Asian Pacific Journal of Tropical Biomedicine*, *5*(3), 182–189.

Samicho, Z., Roha, S., Mutalib, A., and Abdullah, N. (2013). Amino acid composition of droughtmaster beef at various beef cuts. *Agricultural Sciences*, *4*(5), 61–64.

Schmeltz, I., Schlotzhauer, W. S., and Higman, E. B. (1972). Characteristic products from pyrolysis of nitrogenous organic substances. *Beitrage Zur Tabakforschung*, *6*(3), 134–138.

Schut, H. A. J., and Snyderwine, E. G. (1999). DNA adducts of heterocyclic amine food mutagens: Implications for mutagenesis and carcinogenesis. *Carcinogenesis*, *20*(3), 353–368.

Shachman, M. (2004). Brix - Sugar Inversion. In M. Shachman (Ed.), *The Soft Drinks Companion: A Technical Handbook for the Beverage Industry* (pp. 79–81). Washington: CRC Press

Sharma, R. K., Chan, W. G., and Hajaligol, M. R. (2009). Effect of reaction conditions on product distribution from the co-pyrolysis of a-amino acids with glucose. *Journal of Analytical and Applied Pyrolysis*, *86*, 122–134.

Sharma, R. K., Chan, W. G., Seeman, J. I., and Hajaligol, M. R. (2003). Formation of low molecular weight heterocycles and polycyclic aromatic compounds (PACs) in the pyrolysis of a-amino acids. *Journal of Analytical and Applied Pyrolysis*, *66*, 97–121.

Sharma, R. K., Chan, W. G., Wang, J., Waymack, B. E., Wooten, J. B., Seeman, J. I., and Hajaligol, M. R. (2004). On the role of peptides in the pyrolysis of amino acids. *Journal of Analytical and Applied Pyrolysis*, *72*, 153–163.

Shin, H. S., Park, H. Y., and Park, D. (2003). Influence of different oligosaccharides and inulin on heterocyclic aromatic amine formation and overall mutagenicity in fried ground beef patties. *Journal of Agricultural and Food Chemistry*, *51*(23), 6726–6730.

Shrivastava, A., and Gupta, V. B. (2011). Methods for the determination of limit of detection and limit of quantitation of the analytical methods. *Chronicles of Young Scientists*, *2*(1), 21–25.

Singh, L., Varshney, J. G., and Agarwal, T. (2016). Polycyclic aromatic hydrocarbons formation and occurrence in processed food. *Food Chemistry*, *199*, 768–781.

Skog, K. (1993). Cooking procedures and food mutagens: A literature review. *Food and Chemical Toxicology, 31(9),* 655-675.

Skog, K., and Jägerstad, M. (1990). Effects of monosaccharides and disaccharides on the formation of food mutagens in model systems. *Mutation Research*, *230*, 263–272.

Skog, K., and Solyakov, A. (2002). Heterocyclic amines in poultry products: A literature review. *Food and Chemical Toxicology*, *40*(8), 1213–1221.

Skog, K., Solyakov, A., and Jägerstad, M. (2000). Effects of heating conditions and additives on the formation of heterocyclic amines with reference to amino-carbolines in a meat juice model system. *Food Chemistry*, 68, 299–308.

Sugimura, T., Wakabayashi, K., Nakagama, H., and Nagao, M. (2004). Heterocyclic amines: Mutagens/carcinogens produced during cooking of meat and fish. *Cancer Science*, *95*(4), 290–299.

Agency for Toxic Substances and Disease Registry (ASTDR) (1995). *Toxicological Profile for Polycyclic Aromatic Hydrocarbons (PAHs)*. Atlanta: Public Health Service. Retreived 11 Februari 2019 from https://www.atsdr.cdc.gov/toxprofiles/tp69.pdf

Turesky, R. J. (2007). Formation and biochemistry of carcinogenic heterocyclic aromatic amines in cooked meats. *Toxicology Letters*, *168*, 219–227.

Van Boekel, M. A. J. S. (2008). Kinetic Modeling of Reactions in Foods. In M. A. J. S. Van Boekel (Ed.)*, Model and Modeling* (pp. 2[1]-2[12])*.* Florida: CRC Press.

Viegas, O., Novo, P., Pinto, E., Pinho, O., and Ferreira, I. M. P. L. V. O. (2012). Effect of charcoal types and grilling conditions on formation of heterocyclic aromatic amines (HAs) and polycyclic aromatic hydrocarbons (PAHs) in grilled muscle foods. *Food and Chemical Toxicology*, *50*, 2128–2134.

Vitaglione, P., and Fogliano, V. (2004). Use of antioxidants to minimize the human health risk associated to mutagenic/carcinogenic heterocyclic amines in food. *Journal of Chromatography B*, *802*(1), 189–199.

Wang, S. F., Liu, B. Z., Sun, K. J., and Su, Q. D. (2004). Gas chromatographic-mass spectrometric determination of polycyclic aromatic hydrocarbons formed during the pyrolysis of phenylalanine. *Journal of Chromatography A*, *1025*, 255–261.

Wong, D., Cheng, K. W., and Wang, M. (2012). Inhibition of heterocyclic amine formation by water-soluble vitamins in Maillard reaction model systems and beef patties. *Food Chemistry*, *133*, 760–766.

Wongmaneepratip, W., and Vangnai, K. (2017). Effects of oil types and pH on carcinogenic polycyclic aromatic hydrocarbons (PAHs) in grilled chicken. *Food Control*, *79*, 119–125.

Wrolstad, R. E. (2012). Reactions of Sugars. In R. E. Worlsted (Ed), *Food Carbohydrates Chemistry* (pp. 35–47). West Sussex: John Wiley & Sons.

Wu, D. L., and Swain, J. W. (1981). *Cooked flavors for smoking products*. 4,379,464 Retrieved 15 February 2015 from https://worldwide.espacenet.com/publicationDetails/biblio?CC=US&NR=4379464&KC=&FT=E&locale=en_EP

Yoshida, D., Saito, Y., and Mizusaki, S. (1984). Isolation of 2-amino-3-methyl-imidazo-[4,5-*f*]quinoline as mutagen from the heated product of a mixture of creatine and proline. *Agricultural and Biological Chemistry*, *48*(1), 241–243.

Yu, D., Chen, M. S., and Yu, S. J. (2016). Effect of sugarcane molasses extract on the formation of 2-amino-1- methyl-6-phenylimidazo [4,5-b]pyridine (PhIP) in a model system. *Food Chemistry*, *197*, 924–929.

Zelinkova, Z., and Wenzl, T. (2015). The occurrence of 16 EPA PAHs in food – a review. *Polycyclic Aromatic Compounds*, *35*(2–4), 248–284.

Zhang, S., Li, R., Zhang, Y., and Zhao, M. (2020). The effect of solvents on the thermal degradation products of two Amadori derivatives. *RSC Advances*, *10*(16), 9309–9317.

Zochling, S., and Murkovic, M. (2002). Formation of the heterocyclic aromatic amine PhIP: identification of precursors and intermediates. *Food Chemistry*, *79*, 125–134.