UNIVERSITI PUTRA MALAYSIA

EFFECTS OF DIFFERENT TYPES OF OIL AND STORAGE CONDITIONS ON ACRYLAMIDE FORMATION IN SWEET POTATO IPOMOEA BATATAS L. LAM CHIPS

LIM PEK KUI

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By

LIM PEK KUI

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

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DEDICATION

To my beloved husband Chew Tat Sen, for his unfailing support and encouragement.

Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

EFFECTS OF DIFFERENT TYPES OF OIL AND STORAGE CONDITIONS ON ACRYLAMIDE FORMATION IN SWEET POTATO *IPOMOEA BATATAS* L. LAM CHIPS

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January 2014

Chair: Jinap Selamat, PhD
Faculty: Food Science and Technology

Acrylamide, a carcinogen compound is usually generated in carbohydrate-rich processed food during heat treatment. The objectives of this study were firstly to evaluate the precursors of acrylamide formation in sweet potato chips via ten similar consecutive deep-frying experiments with each type of vegetable oil (palm olein, coconut, canola and soya bean) at the temperature of 180 °C for 2 minutes. The second objective was to determine the effect of storage (at 15 °C and 28 °C) of sweet potato roots on acrylamide prescursors, i.e. sugars and free amino acids and correlations of precursors with the acrylamide formation. At each interval of the storage period (at 0, 14, 21, 28, 35 and 42 days in 15 °C; whilst at 0, 5, 10, 15 and 25 days in 28 °C, respectively), sweet potato roots were sliced and deep fried into chips at the temperature of 180 °C for 105 seconds. Sweet potato samples for both first and second objectives were analysed for sugars and free amino acids analysis. The third objective of this study was to assess the influence of the lipids and lipids oxidation by the sweet potato powder model system at 180 °C on acrylamide formation. The role of lipids, reducing sugar and artificial antioxidant (i.e. butylated hydroxytoluene, BHT) in acrylamide formation were also studied. The last objective was to investigate the effects of adding free amino acids (asparagine, glutamine, glutamic acid, aspartic acid, alanine, serine and glycine) on the formation and reduction of acrylamide using sweet potato powder model. For the third and forth objectives, the reactants of the binary and ternary model (asparagine-glucose/fructose/sucrose, asparagine-palm olein/soya bean oil and asparagine-glucose-palm olein/soya bean oil for the third objective; whilst amino acid-glucose and amino acid-asparagine-glucose for the last objective, respectively) were singly homogenized in silica gel 60 by using a ceramic pestle. Additionally, oils sample (palm olein, soya bean oil, soya bean oil with added BHT, coconut oil and canola oil) and free amino acids (asparagine, glutamine, glutamic acid, aspartic acid, alanine, serine and glycine) were singly homogenized in sweet potato powder model system. All models were heated under nitrogen at 180 °C in closed test tube for 20 min in a laboratory oven.

The general linear model (GLM) analysis of variance (ANOVA) was applied to the first objective analysis, whilst the ANOVA and Tukey's multiple comparisons test was also used to assess the significant of differences for the entire study of comparison test. However, Pearson correlation was used in the second objective to assess the relationships of the reducing sugars and acrylamide concentration. Furthermore, multivariate analysis was applied by performing orthogonal partial least squares (OPLS) regression analysis to determine the correlations between the metabolites (glucose, fructose, sucrose, reducing sugars, total sugars, and free amino acids) and acrylamide formation.

The analysis of sugar and reducing sugars was performed by a high performance liquid chromatography (HPLC) equipped with a refractive index (RI) detector. Free amino acids concentrations were determined with an EZ:faast amino acid analysis kit and were analysed by a gas chromatograph (GC) that was equipped with a flame ionization detector (FID). Sweet potato chips were homogenized by a processor and samples were cleaned by Oasis HLB and MCX cartridges before acrylamide analysis was carried out. The acrylamide concentrations were determined by liquid chromatography equipped with a triple-quadrupole mass spectrometer(LC/MS-MS). In addition, an atmospheric pressure chemical ionization (APCI) source was used.

During the deep-frying experiments of sweet potato chips, acrylamide was detected in the range of 296 to 2849 µg/kg. Raw sweet potato roots were also found to contain acrylamide precursors (glucose & fructose, free asparagine). Meanwhile, multivariate data analysis revealed that the sugars (glucose, fructose, sucrose), total reducing sugars, total sugars, and free amino acids (asparagine, serine, alanine, threonine) were positively correlated to acrylamide formation, whilst glycine and valine were negatively correlated to acrylamide formation. Furthermore, the model study confirmed that free amino acids (asparagine, glutamine, glutamic acid, aspartic acid, alanine, serine, glycine) are the precursors of acrylamide formation, and asparagine was the predominant amino acid. The storage experiment showed positive correlation between the concentration of acrylamide and with those of serine and alanine; however, no significant different was found between the treated and the controlled samples with the addition of serine and alanine. The results were probably caused by the synergism by serine and alanine with other chemical compositions in sweet potato roots during storage. On the other hand, there was insignificant acrylamide reduction by gylcine; this was perhaps attributed to the abundant of sugars in the samples; as a result, there was no competition between glycine and asparagine for the available carbonyl compound (in sugars). Therefore, glycine might not be a suitable additive for reducing acrylamide formation in sweet potato products.

Although, lower acrylamide concentration in sweet potato chips was mostly detected by using a frying oil with medium degree of unsaturation (e.g palm olein), no specific oil type was found to be superior; this may be due to oil deterioration after many repeated use of all oil tested in the study for frying. Moreover, the study also indicated that combination of total unsaturated fatty

acid and total oxidation of oil influenced the acrylamide formation positively (r^2 = 0.986, p = 0.000). The study concluded that the lipids should not be ignored in the generation of acrylamide.

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

KESAN PELBAGAI JENIS MINYAK SAYURAN DAN KEADAAN PENYIMPANAN KE ATAS PEMBENTUKAN AKRILAMIDA DI DALAM KEREPEK UBI KELEDEK *IPOMOEA BATATAS* L. LAM

Oleh

LIM PEK KUI

Januari 2014

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

Akrilamida merupakan sebatian karsinogen yang dihasilkan dalam makanan kaya dengan karbohidrat yang diproses semasa rawatan haba. Objektif kajian ini bermula dengan menilai pelopor pembentukan akrilamida dalam kerepek ubi keledek apabila ia digoreng dan disimpan. Pengaruh pelbagai jenis minyak sayuran, iaitu olein minyak sawit, minyak kelapa, minyak canola dan minyak kacang soya telah disiasat dalam sepuluh sesi menggoreng berturut-turut pada suhu 180 °C selama 2 minit. Objektif kedua adalah untuk menentukan kesan simpanan (di 15 ° C dan 28 ° C) daripada akar ubi keledek ke atas pelopor akrilamida, iaitu gula dan asid amino bebas dan korelasi pelopor dengan pembentukan akrilamida juga dikaji. Pada setiap selang tempoh simpanan (pada 0, 14, 21, 28, 35 dan 42 hari dalam 15 °C; manakala pada 0, 5, 10, 15 dan 25 hari dalam 28 ° C, masing-masing), ubi keledek dihiris dan digoreng pada suhu 180 ° C selama 105 saat. Sampel ubi keledek bagi objektif pertama dan kedua telah dianalisa untuk gula dan asid amino bebas analisis. Objektif ketiga kajian ini adalah untuk menilai pengaruh lipid dan kesan pengoksidaan lipid oleh sistem model tepung ubi keledek pada 180 °C kepada pembentukan akrilamida. Peranan lipid, gula penurun dan antioksidan tiruan (iaitu, hydroxytoluene butylated; BHT) dalam pembentukan akrilamida juga telah dikaji. Objektif terakhir adalah untuk menilai kesan penambahan asid amino bebas, iaitu asparagina, glutamina, asid glutamik, asid aspartik, alanina, serina dan glycina kepada pembentukan dan penurunan akrilamida di dalam sistem model tepung ubi keledek. Untuk objektif ketiga and terakhir, bahan tindak balas bagi model binari dan ternari (asparagina-glukosa/fruktosa/sukrosa, asparagina- sawit olein/minyak kacang soya, asparagina-glukosa-sawit olein/minyak kacang soya bagi objektif ketiga; manakala asid amino-glukosa dan asid amino-asparagina-glukosa untuk objektif terakhir, masing-masing) telah diseragamkan secara tunggal dalam gel silika 60 dengan menggunakan mortar seramik.

Analisis gula dan gula penurun telah dijalankan oleh kromatografi cecair prestasi tinggi (HPLC) dilengkapi dengan pengesan indeks biasan (RI). Kepekatan asid amino bebas ditentukan dengan EZ-faast asid amino kit dan dianalisis oleh kromatografi gas (GC) yang dilengkapi dengan pengesan

pengionan nyala (FID). Kerepek telah dihancur seragam oleh pengisar dan sampel telah dibersihkan oleh Oasis HLB dan MCX kartrij sebelum analisis akrilamida dijalankan. Kepekatan akrilamida ditentukan oleh kromatografi cecair prestasi tinggi yang dilengkapi dengan spektrometer jisim tiga Quadrupole (LC/MS-MS). Di samping itu, tekanan pengionan kimia (APCI) sumber atmosfera telah digunakan.

Semasa kajian menggoreng, akrilamida telah dikesan dalam kerepek ubi keledek dalam lingkungan 296 hingga 2849 μg/kg. Ubi keledek mentah didapati mengandungi pelopor akrilamida, yang merupakan gula penurunan, iaitu glukosa dan fruktosa, dan asparagina bebas. Analisis data multivariat menunjukkan bahawa gula, iaitu glukosa, fruktosa, sukrosa, jumlah gula penurunan, jumlah gula, dan asid-asid amino bebas, iaitu asparagina, serina, alanina, dan treonina telah dikaitkan secara positif kepada pembentukan akrilamida, manakala glycina dan valina dikaitkan secara negatif kepada pembentukan akrilamida. Tambahan pula, kajian model menunjukkan bahawa asid amino bebas, iaitu asparagina, glutamina, asid glutamik, asid aspartik, alanina, serina dan glycina adalah pelopor pembentukan akrilamida dan asparagina merupakan asid amino penyumbang utama kepada pembentukan akrilamida. Walaupun ujian penyimpanan menunjukkan korelasi positif antara kepekatan akrilamida dengan serina dan alanina, tiada perubahan ketara berbanding dengan kawalan diperhatikan apabila penambahan kedua-dua asid amino dalam kajian model. Ini mungkin disebabkan oleh kesan sinergistik oleh kedua-dua asid amino ini dengan komposisi kimia yang lain dalam ubi keledek semasa penyimpanan. Pengurangan akrilamida yang tidak ketara oleh gylcina mungkin berpunca daripada kandungan gula yang tinggi dalam sampel di mana tiada persaingan antara glycina dan asparagina untuk sebatian karbonil (gula) yang didapati. Lantarannya, glycina mungkin bukan satu bahan tambahan yang sesuai untuk mengurangkan pembentukan akrilamida dalam produk-produk ubi keledek.

Walaupun, akrilamida yang lebih rendah didapati dalam kerepek ubi keledek yang digoreng dengan minyak berketaktepuan sederhana, iaitu olein sawit. Namun tiada minyak yang terunggul selepas banyak kali digunakan untuk menggoreng, disebabkan oleh kemerosotan minyak. Di samping itu, kajian model menunjukkan bahawa gabungan jumlah asid lemak tak tepu dan jumlah pengoksidaan mempengaruhi pembentukan akrilamida secara positif (r^2 = 0.986, p = 0.000). Pengaruh yang ketara oleh keadaan minyak sayur-sayuran yang digunakan untuk menggoreng dan pengoksidaan minyak untuk pembentukan akrilamida menunjukkan sumbangan minyak dalam pembentukan akrilamida, tidak patut diabaikan.

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I certify that a Thesis Examination Committee has met on 6 January 2014 to conduct the final examination of Lim Pek Kui on her thesis entitled "Effects of Different Types of Oil and Storage Conditions on Acrylamide Formation in Sweet Potato *Ipomoea batatas* L. Lam Chips" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

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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 Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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DECLARATION

Declaration by graduate student

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