Jurnal Sains Kesihatan Malaysia **16**(2) 2018: 113-117
DOI : http://dx.doi.org./10.17576/JSKM-2018-1602-14

Artikel Asli/*Original Article*

Health Risk Assessment of Acrylamide in Deep Fried Starchy Foods among Students of Kolej Tun Syed Nasir, Universiti Kebangsaan Malaysia

(Penilaian Risiko Kesihatan Berkaitan Akrilamida dalam Pengambilan Makanan Berkanji Gorengan Minyak Penuh oleh Pelajar Universiti di Kolej Tun Syed Nasir, Universiti Kebangsaan Malaysia)

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ABSTRACT

Studies found that deep fried starchy foods may contain potential compound to increase cancer risk known as acrylamide. Identification of acrylamide health risk assessment in deep fried starchy food among students in Kolej Tun Syed Nasir, Kuala Lumpur was investigated. A survey was conducted to obtain data on types of deep fried starchy food preferred by the subjects, the food source, intake frequency and also the serving size. Acrylamide in the food sample was quantified using High Performance Liquid Chromatography (HPLC). Based on this study, the estimated acrylamide intake are 0.042 µg/kg/day for fries, 0.012 µg/kg/day for fried banana, 0.129 µg/kg/day for fried sweet potato and 6.101 µg/kg/day for the coated fried chicken, while the acrylamide level in anchovies' fritter cannot be detected (N/A). Based on the hazard quotient (HQ) calculated, the intake of french fries, fried banana and fried sweet potato do not give a significant health risk (HQ < 1). However, for the coated fried chicken, the HQ value is more than 1 which could possibly cause a health risk. Based on individual excess lifetime cancer risk (IELCR) calculated, the deep fried starchy food intake cancer risk of coated fried chicken is higher (3×10^{-2}) compared to fried sweet potato (5.81×10^{-4}), french fries (1.89×10^{-4}) and anchovies fritter (5.4×10^{-5}). The students' intake of coated fried chicken can give a significant health risk compare to other tested food, however in term of cancer risk, all of the tested food give a high number of probability of increased risk of cancer.

Keywords: Health risk assessment; acrylamide; deep fried starchy foos

ABSTRAK

Kajian mendapati bahawa makanan berkanji gorengan minyak penuh mengandungi bahan berpotensi meningkatkan risiko kanser yang dikenali sebagai akrilamida. Risiko kesihatan terhadap akrilamida di dalam makanan berkanji gorengan minyak penuh dikenal pasti dalam kajian ini. Soal selidik dilakukan bagi mendapatkan jenis makanan berkanji gorengan minyak penuh yang menjadi pilihan pelajar, sumber mereka mendapatkannya, kekerapan pengambilan serta saiz hidangan makanan tersebut. Kandungan akrilamida dalam sampel makanan ditentukan dengan menggunakan High Performance Liquid Chromatography (HPLC) bagi menganggar jumlah pendedahan para pelajar terhadap akrilamida dalam makanan tersebut. Anggaran pengambilan akrilamida oleh pelajar KTSN yang dikaji adalah 0.042 µg/kg/hari bagi kentang goreng, 0.012 µg/kg/hari bagi pisang goreng, 0.129 µg/kg/hari bagi keledek goreng dan 6.101 µg/kg/hari bagi ayam goreng bersalut tepung, manakala kandungan akrilamida dalam cucur tidak dapat dikesan. Melalui pengiraan hazard quotient (HQ) didapati bahawa pengambilan kentang goreng, pisang goreng dan keledek goreng tidak memberikan kesan risiko kesihatan yang ketara (HQ < 1). Namun bagi ayam goreng bersalut tepung, nilai HQ melebihi 1 iaitu mampu memberikan risiko kesihatan yang ketara. Berdasarkan hasil pengiraan risiko pendedahan kanser (IELCR), didapati nilai risiko kebarangkalian terhadap kanser bagi corak pengambilan ayam juga adalah lebih tinggi ($3x10^{-2}$) berbanding keledek goreng (5.81×10^{-4}), kentang goreng (1.89×10^{-4}) dan cucur bilis (5.4×10^{-5}). Kesimpulannya, hanya pengambilan ayam goreng bersalut tepung dalam kalangan pelajar mampu memberikan kesan risiko kesihatan ketara, namun bagi risiko pendedahan terhadap kanser, kesemua makanan dikaji memberikan nilai kebarangkalian peningkatan risiko kanser yang tinggi.

Kata kunci: Penilaian risiko kesihatan; akrilamida; makanan bergoreng tinggi kanji

INTRODUCTION

Acrylamide is a vinyl monomer produced as intermediate through hydration of acrylonitrile (Hussein 2006). It is formed as colourless and odourless crystalized solid and commonly used in cosmetics (Li et al. 2012), construction and as flocking agent in water treatment due to its ability to polymerized (Pedreschi et al. 2014). Acrylamide is also an unsaturated-amide molecules that easily absorbed by the body and easily distributed to the organ such as thymus, liver, heart and kidney after ingestion due to its small molecular size (Capuano & Fogliano 2011).

Acrylamide become one of the worldwide concern due to its association with increasing risk of cancer and it is also one of the toxic component to the body (WHO 2011). Previous studies found out that ingestion of acrylamide through food intake gives a positive association to the increasing risk of ovarian and endometrial cancer in women (Hogervorst et al. 2007).

A study conducted by Swedish scientists in April 2002 detected a significant amount of acrylamide in food that are cooked in high temperature which is more than 120°C except through boiling (Tareke et al. 2002). The same study also found that acrylamide content in starchy food is higher than protein source foods (Tareke et al. 2002). IARC (2014) also stated that one of the main acrylamide exposure in human is through food intake especially deep fried , baked or roasted foods.

Previous study stated that potatoes based food contribute one of the highest acrylamide exposure among European (Freisling et al. 2013) and in Malaysia, a study on banana based snack by Daniali et al. (2010) stated that banana fritters contain a high amount of acrylamide compared to the other banana based snack. A study on medical field students' eating habits in Malaysia found that 73.5% of them consume two or more than two times fried food per week (Ganasegeran et al. 2012). This shows that Malaysian is in high risk of acrylamide exposure through food intake.

Based on United States Environmental Protection Agency (US EPA 2000), risk is a chance to give harm to human health due to the environmental stressor. The stressor refer to chemical, physical and biological hazard that can cause adverse effect. Risk assessment is the scientific evaluation of known or potential adverse health effects resulting from human exposure to foodborne hazards.

Currently in Malaysia, there is no study related to acrylamide health risk assessment. Thus, this study is to determine the acrylamide intake in starchy deep fried food among university students and the health risk assessment since generally most students enjoyed fried food and it is readily available especially at the cafeteria or food stall.

MATERIALS AND METHODS

STUDY DESIGN

A cross-sectional study was conducted on 296 subjects of students that stay in Kolej Tun Syed Nasir, Universiti Kebangsaan Malaysia. The subjects were selected using convenient sampling and excluding those who is smoking and posting out. The sample size was calculated using the known population formula of Krejcie and Morgan (1970).

SURVEY FORM

The survey form consists of two parts; part A and part B. Part A consists of sosio-demographic variables; gender, age, weight and height, while part B consists of information on intake of deep fried starchy food (fries, sweet potatoes and banana fritter); frequency of the intake either per-day, per-week or per-month and the number of servings each time consuming the food. The ethics for this study was approved by the Research Ethic Committee from National University of Malaysia (UKM PPI/111/8/JEP-2016-209).

SAMPLE PREPARATION AND ANALYSIS

Five types of deep fried starchy food sold at the college cafeteria (fries/wedges, sweet potatoes and banana fritter) were selected. These samples (100 g of each sample) were analyzed for detection and quantification of acrylamide using high performance liquid chromatography (HPLC) (Oroian et al. 2015). Reagents used were acrylamide, methanol, acetonitrile, formic acid, n-hexane, potassium hexacyanoferrate 15% (Carrez I) and zinc sulphate 30% (Carrez II) purchased from Sigma Aldrich (Hamburg, Germany). Double deionised water (18 MΩ cm resistivity) produced by a water purification system (Thermofisher, Dreiech, Germany) was used in all solutions. The samples were grounded entirely before being analyzed.

ACRYLAMIDE EXTRACTION

One gram of grounded sample was placed in a 50 ml flask, added with 20 ml water (50°C, to increase acrylamide solubility) and 5 ml *n-hexane* to remove fats in the sample. The flask was then agitated for 30 minutes at 250 rpm using the orbital shaker and centrifuged at 3000 rpm for 20 minutes. The fats layer was removed and 1 ml *Carrez I* and 1 ml *Carrez II* added to remove the protein and purify the water layer. The flask was agitated again for 30 minutes and centrifuged at 3000 rpm for 20 minutes. After centrifugation, the sample formed three layers: upper layer (protein), intermediate layer (acrylamide) and bottom layer (sample sediment). The intermediate layer was purified again using 1 ml *Carrez I* and 1 ml *Carrez II*. The sample once again agitated for 30 minutes at 250 rpm using orbital shaker and centrifuged at 3000 rpm for 20 minutes. The clear layer was removed from the flask, evaporated at nitrogen atmosphere and dried using freeze dryer. The residue was then dissolved in 2 ml water, filtered using 45 μm filter and analyzed using liquid chromatography.

ACRYLAMIDE QUANTIFICATION

HPLC system used was equipped with Diode Array Detector. The wavelength was set at 22 nm and the mobile phase use was pure water (deionise water). The next process and data collection was performed using the LC solution software. A stainless C18 steel analytical column was used. A calibration curve was constructed at 25, 50, 100, 200, 400, 600, 800 and 1000 µg/kg acrylamide solution and the quantification of acrylamide was performed by measurement of peak area at the acrylamide retention time and comparison with the calibration curve. The injection volume was 10µl with 1ml/min flow rate.

CALCULATION OF HUMAN RISK

Rates of daily acrylamide ingestion can be calculated by using the Equation (1):

$$\text{EDI} = \frac{C \times IR \times EF \times ED}{bw \times AT}, \tag{1}$$

where, C is the acrylamide concentration in food obtained from analysis (mg/g), while IR is the ingestion rate of the deep fried food in a day. Based on the survey conducted, it was found that the consumption of potato chips (French fries) was 9.26 g/day, fried banana is 11.64 g/day, sweet potato fries was 8.18 g/day, anchovy fritters 19.47 g/day and coated fried chicken 153.07 g/day. EF is the exposure frequency, 365 days for those who consume deep fried starchy food seven times a week and 52 days for those who consume it once a week. ED is the duration of exposure usually calculated according to the study objective, and is usually calculated for a period of one year or 365 days. AT is the average exposure to the substance is: 365 days/year multiply by ED. While bw is the average body weight. In this study, the average weight of the subjects which is the students who reside in Kolej Tun Syed Nasir was 53.94 kg.

Hazard Quotient used to calculate the health risk and whether the particular risk has significant impact by using Equation (2):

$$\text{HQ} = \frac{EDI}{RfD}, \tag{2}$$

where EDI, intake of heavy metal (as described in previous equation) and *RfD*, reference dose, is an estimated value that recommended by international agencies such as: US EPA, FAO and WHO. It is an estimated safe limit of daily intake for a chemical. The *RfD* of acrylamide by US EPA is 0.002 mg/kg/day. There is no health risk effect if the HQ value is less than 1.

Individual excess lifetime cancer risk was used to calculated the health risk related to carcinogenic compund by using the Equation (3):

$$\text{Risk} = I \times SF, \tag{3}$$

where, I is the exposure assessment and SF is the slope factor for the carcinogenic compound. US EPA has set a slope factor for oral acrylamide exposure is 4.5 (mg/kg/day)$^{-1}$.

STATISTICAL ANALYSIS

Statistical Package for Social Science (SPSS) version 22.0 was used to analyse the data. Kolmogorov-smirnov test was used to determine the normality since the sample size is more than 100 subjects. Descriptive test was used to determine the mean and standard deviation for the acrylamide intake among the subject and to determine acrylamide level in the food samples. Pearson's Chi-squared test was used to test for the food source, top 5 food chosen, intake frequency and serving size. The significant level was set at $p < 0.05$.

RESULTS AND DISCUSSION

CONCENTRATION OF ACRYLAMIDE IN DEEP FRIED STARCHY FOODS

Acrylamide content in banana obtained from this study (400 µg/kg) is in the range of acrylamide content in fried bananas based on the study conducted by Daniali et al. (2010) which found that the content of acrylamide in fried bananas were in the range of 74.0 to 746.8 µg/kg. For the sweet potato, the values obtained (5950 µg/kg) exceeds the range of the content of acrylamide in sweet potatoes that are listed by the WHO (2002), which is in the range of 170-2287 µg/kg with an average value of 1312 µg/kg.

Acrylamide concentration of French fries in this study (1720 µg/kg) is also within the range of the content of acrylamide by other studies. Tareke at al. (2002) found that the acrylamide content in the sample chips (French fries) is in the range of 224-3700 µg/kg. Studies by Oroian et al. (2015) found that the acrylamide content in French fries from the supermarket has a range of 210-2922 µg/kg with an average concentration of 783 µg/kg. WHO (2005) also lists the contents of acrylamide in French fries is in the range 59-12800 µg/kg.

The concentration of acrylamide in coated fried chicken was 2150 µg/kg also exceed the values listed by WHO (2002) for white meat coated with bread crumbs which is between 39-64 µg/kg with an average value of 52 µg/kg. While acrylamide content in anchovy fritters cannot be detected was likely due to the concentration which is too low.

The difference in value of acrylamide in the food samples that were compared to previous studies may be influenced by several factors such as used cooking oil (Capuano et al. 2010; Ehling et al. 2005; Lim et al. 2014), the frying duration and temperature used for frying (Leung et al. 2003; Luning & Sanny 2016; Zhang et al. 2015). The summary of the acrylamide content analysed were listed in Table 1.

HUMAN RISK ESTIMATION

Data obtained from the survey and food samples analysis were used to calculate the acrylamide EDI value of the

TABLE 1. Acrylamide content in deep fried food

Food Sample	Acrylamide content/μgkg^{-1}
Sweet Potato	5950
Coated Fried Chicken	2150
French fries	1720
Fried banana	400
Anchovies fritter	N/A

subjects. The EDI value was then used to calculate the risk estimation (Equation 1). Based on the calculation it is found that the EDI of acrylamide for sweet potato (1.29×10^{-4} mg/kg/day), French fries (4.2×10^{-5} mg/kg/day) and fried banana (1.2×10^{-5} mg/kg/day) is less than the reference dose while the EDI of coated fried chicken (6.101×10^{-3} mg/kg/day) exceeds the reference dose (0.002 mg/kg/day).

From the calculation of Hazard Quotient, HQ (Equation 2), the results indicated that HQ for coated fried chicken is more than 1 (3.05) while the other food sample's HQ are less than 1 which is 0.06 for sweet potato, 0.02 for French fries and 0.006 for fried banana. The HQ value is used to estimate potential health risk or effect. HQ value exceeding 1 means that there is probability that high potential health effect to occur.

Acrylamide is a carcinogen compound. Thus, the more accurate way to assess the health risk is by using the individual excess lifetime cancer risk (IELCR) (Equation 3), where the EDI value times with the slope factor value. US EPA has set the slope factor of acrylamide through oral exposure is 4.5 (mg/kg/day)$^{-1}$. Based on the calculation, it is found that the acrylamide cancer risk value for fried chicken is the highest compare to the other foods sample which is an extra lifetime cancer risk of 3×10^{-2} (corresponding to 3 cancer cases per 100 individuals) followed by sweet potato which is 5.81×10^{-4} (corresponding to 581 cancer cases per 1000,000 individuals), French fries which is 1.89×10^{-4} (corresponding to 189 cancer cases per 1000,000 individuals) and lastly fried banana which is 5.4×10^{-5} (corresponding to 54 cancer cases per 1000,000 individuals). The EDI, HQ and IELCR value for acrylamide in the deep fried food samples were calculated and summarized in Table 2.

CONCLUSION

In conclusion, the acrylamide level varies between the food samples studied. Intake of coated fried chicken was found to give a more significant health risk effect compared to the other food samples (sweet potato, fried banana, french fries and anchovies fritter) with a HQ value of 3.05 (HQ > 1) and cancer risk of probable 3 extra lifetime cancer risk per 100 individuals.

TABLE 2. Summary of acrylamide estimated daily intake (EDI), Hazard Quotient (HQ) and Individual excess lifetime cancer risk (IELCR)

Food Sample	EDI (mg/kg/day)	HQ	IELCR
Sweet Potato	1.29×10^{-4}	0.06	5.81×10^{-4}
Coated Fried Chicken	6.101×10^{-3}	3.05	3×10^{-2}
French fries	4.2×10^{-5}	0.02	1.89×10^{-4}
Fried banana	1.2×10^{-5}	0.006	5.4×10^{-5}
Anchovies fritter	N/A	N/A	N/A

ACKNOWLEDGEMENT

We would to thank research grant funding from Universiti Kebangsaan Malaysia (GP-K015172) that covers part of this work.

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Received: December 2016
Accepted for publication: March 2018