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Determination of acrylamide in banana based snacks by gas chromatography-mass spectrometry

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Abstract: Fried and baked banana-based snacks are popular in South East Asia and banana chip is popular in other countries, such as India, Indonesia, China, African countries, etc; these snacks may contain acrylamide in concentration which may be of concern due to its toxicity. This study was carried out to determine acrylamide concentration in popular banana based snacks in Malaysia using a modified method of gas chromatography-mass spectrometry. The limit of detection and limit of quantitation of the modified method are 5 and 15 µg/kg, respectively. Acrylamide concentration of five types of Malaysian popular fried and baked banana based snacks from different local markets ranged 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*), not detected 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 a significant difference (P<0.05) in acrylamide concentration between different food types. From the estimate of banana fritter consumption data, the highest exposure to acrylamide in Malaysia is 1.2 µg/kg body weights.

Keywords: Acrylamide, gas chromatography-mass spectrometry, Banana-based snacks

Introduction

Acrylamide is probably carcinogenic to humans (Group 2A), classified by the International Agency for Research on Cancer (IARC, 1994). Acrylamide is also considered as a potential genetic and reproductive toxin with mutagenic and carcinogenic properties in experimental mammalians in vitro and vivo study (Dearfield *et al*., 1995; Dearfield *et al*., 1988). Meanwhile, the risk assessment of acrylamide assessed by the Scientific Committee on Toxicity Ecotoxicity and the Environment (CSTEE) of the European Union (EU) confirmed that the exposure of acrylamide to humans should be kept as low as possible with regard to the inherent toxic properties of acrylamide (CSTEE, 2001). It has also been reported to be a neurotoxin (Dearfield *et al*., 1988). Appropriate laboratory safety precautions should be used when working with this chemical. It is stable in acid, decomposes in base, and is sensitive to light (U.S. EPA, 1994).

Rydberg *et al*. (2003) showed that, higher temperature (200°C) combined with expanded heating times produced reduced levels of acrylamide, due to elimination/degradation processes. They found the presence of asparagine or monosaccharides (in particular; fructose, glucose and glyceraldehyde) to increase the net content of acrylamide. The dependence on pH of the acrylamide content exhibited a maximum around pH 8, in particular, lower pH was shown to enhance elimination and decelerate formation of acrylamide. In contrast, the effects of additions of antioxidants or peroxides on acrylamide content were small or nonexistent.

The desirable colors as well as the relatively fast creation of unique flavors and texture of snacks were easily developed by deep-frying process; however acrylamide was mostly found in these tasty foodstuffs (Pedreschi *et al.,* 2007). Color is one of the most important food product characteristics as it is the first quality parameter evaluated by the consumer, even before the food enters the mouth (Pedreschi *et al*., 2005). The sugar concentrations which could be influenced by pre and post-harvest factors, determined the acrylamide formation (De Wilde *et al*., 2006). It is known that the formation pathway

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of this compound is linked to the Maillard browning reaction in which reducing sugars and amino acids are decisive precursors (Stadler *et al*., 2003). In addition, the degree of acrylamide formation during Maillard browning reaction was very much determined by the free asparagine relative to the reducing sugar concentration in foods (Amrein *et al*., 2003).

Gas chromatography- mass spectrometery (GC-MS) is one of the most sensitive methods for analysis of acrylamide. It has been used to determine acrylamide concentrations in different foodstuffs (Pittet *et al*., 2003). To analyze acrylamide, there is a need to use bromine water as a derivatization agent. Bromine vapor is released from bromine water solutions when they are open to the air. This vapor is harmful if inhaled. The study used a modified method with the reduction in the usage of bromine during derivatization of acrylamide and the introduction of the usage of SPE cartridges in acrylamide extraction.

Banana and plantain constitute fourth world rank of the most significant foodstuffs after rice, corn and milk (FAO, 1999; INIBAP, 2001). Fried and baked traditional banana based foods, such as banana fritter (*pisang goreng*), banana chips (*kerepek pisang*), sweet banana chips (*kerepek pisang manis*) and banana balls (*cekodok pisang*) are popular snacks in Malaysia. These foods may contain acrylamide at concentrations which is dangerous to health. Acrylamide ($C_3H_3ONH_2$) is a chemical substance that is formed naturally in carbohydrate and protein rich foods when they are cooked at high temperatures (Pedreschi *et al*., 2007). To our knowledge, there is no study on acrylamide concentration in Malaysian banana based foods. Hence the objective of the study was to determine the concentration and estimated daily intake of acrylamide in these snack foods.

Materials and Methods

Instrumentation and Reagents

Gas chromatography-mass spectrometry (GC-MS) used was HP 5890 gas chromatograph, equipped with a HP 5973 mass spectrometer and HP 7683 auto sampler (Hewlett-Packard, Avondale, PA). All chemicals used were of analytical grade or above. Isotope [$^{13}C_3$] acrylamide (isotopic purity, 99%) was obtained from Cambridge Isotope Laboratories (Andover, MA, USA), whereas acrylamide (standard for GC, assay ≥ 99.8%) was from Sigma-Aldrich (Hong Kong, China). *N*-hexane, potassium bromide, sodium thiosulfate, and sodium sulfate anhydrous were all obtained from Merck (Darmstadt, Germany) and were of analytical grade. Triethylamine was purchased from Fluka (Buchs, Switzerland) whereas ethyl acetate was supplied by SDS (Peypin, France). Oasis MCX 3 cc (60 mg) liquid phase and Oasis HLB 6 cc (200 mg) solid phase extraction cartridges were purchased from Waters Corporation (Massachusetts, USA).

Sample collection

Three samples of each snack from five locations were obtained from local market in Selangor, Malaysia. Snack samples included banana fritter *(pisang goreng*), banana chips (*kerepek pisang*), sweet banana chips (*kerepek pisang manis*), banana cake (*kek pisang*) and banana balls (*cekodok pisang*).

Acrylamide extraction

Acrylamide was determined as a 2-bromopropenamide derivative following the method by Pittet *et al*., (2004) and Young *et al*. (2004) with some modifications. One hundred gram of crushed snack was weighed and then mixed (Braun multiquik ZK3, Frankfurt, Germany) with 100 mL of distilled for 2 minutes 2g of the mixture was then mixed with 3mL of 2 M NaCl and transferred into a centrifuge tube. The mixture was then homogenized by the Ultra-Turrax (IKA-T25 Basel, Switzerland) for 30 sec. 100µL of internal standard was then added immediately and was shaken by auto-vortex mixer (Stuart scientific, Manchester**,** England) for 1 min. The tube was centrifuged in a refrigerated centrifuge (3-18K, Sigma, Gillingham Dorset, UK) at 10956 RCF(x g) for 30 min; the aqueous layer was filtered through glass wool (the fat layer remained on glass wool). The SPE columns (HLB, MCX) were conditioned with 3mL methanol followed by 3mL deionized water for equilibrating; then the extract was passed through the cartridges being eluted by 3 mL methanol followed by 3 mL deionized water. Cartridge extract was made up to 50 mL with deionized water.

Potassium bromide and sodium sulfate anhydrous were dried by calcination in a crucible at 600°C using muffle furnace (Carbolite Furnaces; Esser, England) for 6 hours and later stored in tightly closed container. 7.5 g calcinated potassium bromide was dissolved into the cartridge extract with stirring, and the pH of the solution was adjusted 2 by the addition of 15 drops of hydrobromic acid (47%). 2.5 mL of saturated bromine–water solution was then added to the amber flask whilst stirring. The flask was transferred into an ice bath for overnight. After the reaction was completed, the excess bromine was decomposed by adding 5 drops of 0.7 M sodium thiosulfate solution until the yellow color disappeared, then 10 g of

calcined sodium sulphate (anhydrous) were added and stirred vigorously for 5 min using a magnetic stirrer.

The mixture was transferred to a 250mL separating funnel and extracted with 20mL of ethyl acetate/ hexane (4:1, v/v) by shaking for 10 min at medium speed (ca. 255 pulses/ min) followed by phase separation using Recipro shaker (RS-1, Jeio Tech Co., Gyeonggi-do, Korea). The organic phase was poured into a centrifuge tube contain 4 g calcinated sodium sulfate. This step was repeated twice using 20mL ethyl acetate/hexane (4:1, v/v). The mixture was centrifuged at 10956 RCF (x g) for 30 min and decanted through glass wool into 50mL round-bottom flask and then dried using rotary evaporator (Rotavapor R-210, Buchi Labrortechnik AG, Flawil, Switzerland). The final dried residue was re-dissolved in 50 µL of triethylamine and 450 µL of ethyl acetate and transferred into an amber vial and was stored in a freezer at –20°C until GC-MS analysis.

GC-MS Analysis

The sample extract was injected into GC-MS using Innowax capillary column, 30 m×0.25 µm i.d. and 0.25 µm film thickness (Agilent Technologies, Palo Alto, CA, USA), and helium carrier gas (purity 99.99%) at 1.6 ml/min. Following injection, the column was held at 65°C for 1 min, then programmed at 15°C/min to 170°C, 5°C/min to 200 °C, followed by 40°C/min to 250°C, and held for 15 min at 250°C (total run time: 30/min). Injection was done by an auto sampler that was in split mode (split flow 60 mL/min) with purge activation time of 1 min and injection temperature of 260 °C. The GC–MS interface transfer line was held at 280 °C. Under these conditions, the retention time of acrylamide and ($^{13}C_3$) acrylamide derivatives was 11.3 min. Ions monitored were *m*/*z* 149 for 2-bromopropenamide, and *m*/*z* 154 for 2-bromo ($^{13}C_3$) propenamide by EI mode at 70eV with ion source temperature as high as 230 ºC. The acrylamide quantification was performed using calibration curves.

Estimated daily intake

The estimated daily intake (EDI) values of acrylamide by an adult were calculated using the average values by each type of snacks, i.e. EDI (µg/ kg body weight) = mean concentration of acrylamide (µg/kg) multiplied by the amount of snack consumed day/g and divided by the average weight of an individual (50 kg) (Dybing and Sanner, 2003; Ikema and Egiebor, 2005). The snacks consumption data were obtained from a survey conducted by Jing (2008, thesis unpublished data).

Statistical analysis

Descriptive statistics including minimum, maximum, mean and standard deviation (SD) were measured using Minitab (Release 14 for Windows, Pennsylvania, USA). Analysis of variance at 5% confidence, concentration ($P < 0.05$) and Tukey's test was performed to determine the differences among snacks and locations.

Results and Discussion

A modified method was developed for acrylamide determination in high carbohydrate-protein snacks and banana based snacks were chosen as sample matrices. Table 1 shows the recovery ranges among the analyzed snacks. Limit of detection (LOD) and limit of quantitation (LOQ) were 5 and 15µg/g, respectively. The linear equation and correlation coefficient were Y=6E-06X-7E-06 and 0.9937, respectively. Previous analysis results reported by Ahn *et al*. (2002) showed that the LOD for acrylamide detection in toasted bread, fried chips, grilling and baking potatoes using GC-MS was 25 µg/kg, whereas Tateo *et al*. (2007) found LOD of 25 and 75 µg/kg, respectively and recovery of between 79-85% in 27 samples from fast food restaurants (Ahn *et al*., 2002; Tateo *et al*., 2007). However our study produced relatively lower LOD and LOQ of 5 and 15 µg/kg, respectively and good extraction efficiency with recovery of 84-110%. This indicates that the method was appropriate to be used.

The range and mean value of acrylamide concentration of banana fritter, banana balls, sweet banana chips, banana chips and banana cake are presented in Table 2. The analysis results showed that acrylamide was detected in most samples. The minimum concentration of acrylamide was found in banana cake (29.0 µg/kg) whereas the maximum one was in banana fritter (7468.80 µg/kg). Except for the banana fritter (3,584.8 µg/kg), the mean concentration of acrylamide found in banana snacks was relatively low (74.7, 111.3, 274.79, 267.9 µg/ kg for banana cake, banana chips, banana balls and sweet banana chips, respectively). This could be due to high concentration of reducing sugar ranged from 0.1% to 26.6%, asparagine and glutamine which were at 0.78 and 0.95% dry matter in banana, respectively. (Fernandes et al., 1979).

The concentration of acrylamide obtained in this study was quite low in all samples except for the banana fritter, when compared to potato chips and French fries. This may be due to the fact that the duration of time and temperature for processing of banana chips

Table 1. Recovery of acrylamide determined by GC-MS in different matrices at three different concentrations

Types of snack	Acrylamide added (ng/g)	%Recovery Mean ± SD
Banana Chips (*Kerepek Pisang*)	50	108.3±1.1
	100	96.9±1.0
	200	90.1±1.8
Fritter Banana (*Pisang Goreng*)	100	109.0±0.8
	200	85.1±4.1
	4000	84.0±3.9
Sweet Banana Chips (*Kerepek Pisang Manis*)	50	109.9±0.9
	100	94.7±1.9
	200	91.8±3.2
Banana Cake (*Kek Pisang*)	50	105.1±2.5
	100	95.0±1.7
	200	85.0±4.1
Banana Balls (*Cekodok Pisang*)	50	100.1±1.4
	100	91.5±1.7
	200	88.4±5.4

Table 2. Acrylamide concentrations (µg/kg) in banana based snacks collected from 5 locations

Sample's Location	Location 1	Location 2	Location 3	Location 4	Location 5
Range (BF)	74.0 - 77.5	3409.5 - 3412.5	3632.6 - 3636.6	3347.3 - 3350.8	7466.1 - 7468.8
Mean (BF)	75.6±1.5[a]	3410.5±1.4[b]	3634.8±1.7[c]	3349.0± 1.5[d]	7454.2±1.1[e]
SD (BF)	1.5	1.4	1.7	1.5	1.1
Range (BB)	31.7 - 35.8	62.6- 65.5	169.9 - 173.7	604.9 - 609.1	495.1 - 498.8
Mean (BB)	33.5±1.7[a]	64.1±1.2[b]	171.4±1.6[c]	607.5±1.7[d]	497.4±1.8[e]
SD (BB)	1.7	1.2	1.6	1.7	1.8
Range (SBC)	I90.2 – 193.1	185.7 - 188.2	160.7 - 164.9	302.3 - 305.9	498.0 - 500.4
Mean (SBC)	191.9±1.2[a]	186.9±1.0[b]	162.7±1.7[c]	304.0± 1.4[d]	499.3±1.0[e]
SD (SBC)	1.2	1.0	1.7	1.4	1.0
Range (BCh)	89.8 - 91.3	42.7 - 45.9	28.9 - 32.5	240.9 -243.7	148.1 - 151.0
Mean (BCh)	90.7±0.6[a]	44.0±1.3[b]	30.6±1.6[c]	242.2±1.1[d]	149.4±1.1[e]
SD (BCh)	0.6	1.3	1.6	1.1	1.1
Range (BCa)	29.0- 31.7	152.5-155.4	38.8 - 41.1	ND	ND
Mean (BCa)	30.3±1.2[a]	154.0±1.2[b]	39.8±0.9[c]	ND	ND
SD (BCa)	1.2	1.2	0.9	ND	ND

is not high enough for high acrylamide formation. The highest mean of acrylamide concentration, 3,584.8µg/kg was in banana fritter, which is produced under high temperature processing condition, in the range of 170-190°C. This is in accordance with the finding that acrylamide formation from amino acids and reducing sugar can occur in a solid mixture of the compounds during heating up to 190 °C (Robert *et al*., 2004). Acrylamide concentration in different food product groups in different countries was reported by FAO/WHO. The report indicated acrylamide concentration of 50 – 3500 µg/kg in chips and potato, 30 – 3200µg/kg in biscuits, crackers, toast and bread crisps, 30 – 1346µg/kg in breakfast cereals, 170–230µg/ kg in coffee powder, 30 – 39 µg/kg in fish and seafood products (crumbed and battered) from Norway, Sweden, Switzerland, the United Kingdom and the United States of America (FAO/WHO, 2002.).

Banana is a good source for reducing sugar especially glucose and fructose. It also contains amino acid, mainly glutamine and asparagine (0.95 ± 0.02 and 0.78± 0.01 % dry matter, respectively); the sucrose concentration however, is lower than that of glucose and fructose in ripened banana (Fernandes *et al*., 1979).

One-way ANOVA showed that there was a significant difference ($p<0.05$) between acrylamide concentrations in different banana snacks being studied. This difference is likely due to the different processing methods, temperatures, color, raw materials and repeated usage of frying oil and maturity stage of banana. High oil temperatures during frying significantly increase acrylamide formation (Fiselier *et al*., 2006).

Duration of frying time in banana fritter is higher then the other type of snacks; furthermore repeated usage of cooking oils can also affect on acrylamide formation. Previous investigations on reactions associated with the formation of acrylamide revealed that the process is initiated with the reaction between reducing sugar and amino acid and temperature of processing which indicate that acrylamide might be a product of the Maillard reaction (Mottram and Wedzicha, 2002; Friedman, 2003). The variation among the location can be explained by different chemical composition of the raw materials (the variety and ripeness of banana).

The estimated daily intake of acrylamide in the studied snacks is presented in Table 3. The highest estimated exposure of acrylamide from the consumption of banana snack was due to the banana fritter of (1.2µg/kg body weight) based on the consumption data of 16.69 g/day (Jing, 2008), average concentration of acrylamide found in this study (3584.8ng/kg) and body weight of 50kg. A study in Belgium revealed a median intake of 4µg/kg bw/day, which comprised of biscuits (35.4%), French fries (29.9%), bread (23.5%) and chocolate (11.2%) as the main sources of dietary acrylamide. Foodstuffs consumed in between the three main meals of the day (so called snack type foods) contributed the most to the intake (42.2%).Health risks of the general population are based on an average exposure to 1µg/kg bw/day and increasing to 4µg/kg bw/day for high exposure consumers (Dybing and Sanner, 2003). Even if there are considerable uncertainties in the exposure assessment of acrylamide in food, the safety margins with respect to neurotoxic effects of acrylamide are judged to be large enough to conclude that the risks appear to be very low (Dybing and Sanner, 2003). The exposure could be lower in an intervention group which received free portions of fruit and vegetables, indicating that a nutritionally balanced diet may contribute to a decreased acrylamide intake (Mestdagh et al., 2007).

Daily mean intakes of acrylamide present in foods and coffee in a Norwegian exposure assessment study have been estimated to be 0.49 and 0.46µg/kgbw in males and females, respectively (Dybing and Sanner, 2003). The calculated average acrylamide intake ranges from 0.3 to 0.6 mg/kg body weight per day for

Table 3. Estimated daily intake (EDI) of banana based snacks in Malaysia

Snack types	Consumption[1] (mg/day)	Acrylamide (µg/kg)	EDI (µg/kg bw)
Banana fritter	16.69	3584.8	1.2
Banana balls	11.45	274.7	0.06
Banana chips	5.5	111.3	0.01
Sweet banana chips	NA	267.9	NA
Banana cake	NA	74.7	NA

[1](Jing, 2008).

adults, while children and adolescents tend to ingest more acrylamide on a per bodyweight basis (0.4–0.6 mg/kg). This can be ascribed to a combination of children higher caloric intake as well as their higher consumption of certain acrylamide-rich foods, such as french fries and potato crisps (Dybing *et al*., 2005). However, no significant association was found between acrylamide exposure and risk of prostate cancer concentration; nevertheless, acrylamide adducts to hemoglobin and FFQ-measured acrylamide intakes were moderately correlated (Wilson *et al*., 2008).

Conclusion

Concentration of acrylamide in banana fritter (*Pisang goreng*) is quite high as compared to other types of snack being studied; this maybe due to reuse of oil, duration of frying, and different dough formulation.

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