Volume : 4 | Issue : 10 | October 2015 • ISSN No 2277 - 8179

Research Paper

Acrylamide Content in Selected Commercial Foods in Egypt Using High-Performance Liquid Chromatography–Photodiode Array Detection.

Agriculture

KEYWORDS : Acrylamide, high- performance liquid chromatography, commercial food

Enas Mahmoud Mekawi

Agricultural Biochemistry Department, Faculty of Agriculture, Research Park (Biotechnology Lab.), Benha University, Egypt.

ABSTRACT

This study reports the results of the survey study on acrylamide levels in selected foods products collected randomly from supermarkets in Egypt. Acrylamide (AA) can be industrially produced or formed in processed food by a Millard type of reaction between sugar molecules and the amino acid asparagine. In order to estimate the quality of these foods and their effects on public health, the content of acrylamide in commercial potato chips, breakfast cereals, snacks, biscuit, bread, coffee and baby food has been determined. High-performance liquid chromatography (HPLC) coupled to photodiode array detector technique was employed for the analysis. Extraction method was followed in order to allow low concentrations of acrylamide to be detected. Limit of detection was 30 µg/kg. In general, the highest value of acrylamide (1226 µg/kg) was detected in potato crisps whereas the lowest value was detected in powdered baby food (20 µg/ kg). The outcome of current study has strongly recommended the necessity to conduct a large-scale survey to evaluate the levels of acrylamide in commercial food products.

Introduction:

Acrylamide is potentially toxic classified as a neuro toxicant, carcinogen and teratogen (IARC, 1994). Acrylamide (AA) is an odorless crystalline solid at room temperature, with molecular formula C_3H_5NO and molecular weight of 71.08. The monomeric form of AA is a water-soluble powder and it is employed in different chemical and industrial processes. It is a vinyl monomer and its industrial application is associated with pollution and health risks (Brown *et al*., 1980). Acrylamide is a component of polymers and co-polymer materials used in gel chromatography and in waste water management (Kopp and Dekant, 2009, Bekas *et al.,* 2006). Acrylamide is also a contaminant in foods prepared at very high temperatures. Acrylamide is formed during high-temperature cooking of animal feed and that is found in common human foods that are prepared by cooking at high temperatures has generated interest in the neurotoxicity of dietary exposure to acrylamide. Acrylamide is largely derived from the heat-inducing reactions between the amino group of the amino acid asparagine and carbonyl groups of glucose and fructose in plant-derived foods. The remarkably high levels of acrylamide were observed for the first time by in unexpectedly high levels in heated mashed fried potato (Eriksson, 2005). Direct consumer exposure to acrylamide may result from ingestion of high-carbohydrate foods such as potato crisps and chips, roasted cereals and breads. In Egypt, there has been an upward trend in consumption of snacks, that as fast foods and drinks, between main meals. (Elhassaneen and Sayed, 2015).

At present, several analytical methods are available for determining acrylamide in foods and the majority are classical methods based on high performance liquid chromatography (HPLC) or gas chromatography (GC) techniques (Singh and Balaji, 2010). But, analysis of acrylamide in food products focuses primarily on chromatographic methods such as HPLC–MS and GC–MS (LC-MS) (Jio, *et al.,* 2005; Hoenicke, *et al*., 2004). The HPLC method has the advantage that the transfer of acrylamide into an organic solvent or the removal of water after aqueous extraction is not necessary. HPLC has been credited with qualitative and quantitative analysis of various components, biological and non-biological (Matuszewski *et al*., 2003; Wenzl *et al*., 2003). Reversed phase (RP)-RP-HPLC-method may be used as a convenient, reliable and low-cost alternative system for liquid and gas chromatography coupled to mass spectrometry methods and can be easily adopted by non-specialized analytical laboratories (Michalak *et al*., 2013). Unfortunately, there is a dearth of information concerning the quality of these foods and their effects on public health. Therefore, the present study was carried out to determine the concentrations of acrylamide in selected snacks, cereals, bread products and baby food at available markets in Egypt by Photodiode Array (PDA) HPLC.

Materials and Methods:

Chemicals:

Acrylamide (99%) was purchase from Sigma (MO, USA). Acetone, hexan and acetonitrile (min. 99%) for HPLC analytical grade were purchased from El Gomhouria Company for Trading Chemicals and Medical Appliances, Egypt.

Market Samples:

Samples i.e. potato chips, snacks, cereals, baby food and coffee (different brands), which represent the most common brands distributed in the Egyptian local markets, were collected at random from the Qalyoubia governorate, Egypt. All samples were purchased on January-2015, transported to the laboratory and used for acrylamide determination.

Twenty of various foodstuffs divided into seven groups, were analyzed in this study. Group 1: Potato chips 1 and 2 (according to different producers). (a) Potato chips 1(flavored salt and tomato). (b) Potato chips 2 (flavored pepper and tomato), (c) Potato crisp. Group 2: Maize snacks (flavored cheese and pepper); Group 3: breakfast cereals; Group 4: Bread (a) Arabian white bread, flat, produced from white wheat flour (zero type extraction rate 72%). (b) Arabian dark bread (whole flour is used). (c) Crisp bread (olive and butter); Group 5: Biscuit; Group 6: Coffee (a) Soluble Coffee, (b) Roasted Turkish coffee (Arabica) and Group 7: (a) Powdered baby food, were basically composed of cereal and milk in addition to fruit or honey, (b) Jarred baby food 1 and 2.

Processing and Extraction of Samples:

Samples (potato chips, cereals, crisps) were crushed in a mill cup blender and made homogeneous. Bread and jarred baby food have to be pre-dried carefully using vacuum oven. The sample material was mixed well and then filled into a sample cup.

Extraction was carried out according to the method of Khoshnam *et al*., 2010. Finely ground and homogenized chips sample (5.0 g) were weighed into a closed flask, defatted twice by adding 10 ml hexane and shaking for 5 min. The mixture was dried under vacuum, after decantation for the extraction of AA, 20 ml of acetone and 100 ml of water were added to the defatted sample. The flask was placed in an ultrasonic bath at 40 °C for about 20 min. The acetone was filtered through a filter paper. Gently, 10 ml of the filtrate was evaporated under vacuum to dryness. Then, 2 ml of water was added and shaken thoroughly to dissolve the residue. The aqueous solution was filtered through a disk filter and subjected to HPLC analysis. For baby food, sample were homogenized and different concentrations of AA standard, was used as spiked samples.

Volume : 4 | Issue : 10 | October 2015 • ISSN No 2277 - 8179

Identification and Quantitation of Acrylamide:
Using reversed phase (RP) C18-type column (Hypersil ODS C18) of 4.6 mm × 15 cm. Chromatographic analyses were carried out using a Young Lin HPLC, series YL-9100, equipped with a quaternary pump, an autosampler (YL9150), a degasser, and a YL-9160 spectrophotometric detector (Photo Diode Array detector – PDA), which was set at 202 nm. The flow rate was fixed to 0.5 ml/min. The solvent system consisted of acetonitrile /H2O (70:30). Mode of the HPLC instrument was isocratic and injection volume was 20 µl.

Results and Discussions:
Acrylamide determined in selected commercial samples in 2015. Different methods were reported for the extraction of acrylamide. These could be divided into two main categories extraction with water or with an organic solvent (Khoshnama *et al*., 2010). With respect to the applied HPLC method, in all cases the extracted AA was finally transferred to an aqueous phase prior to injection. Acetone was used to extract the AA from samples. Acetone yield much more clear extract than that of water. In addition, it can be easily evaporated under a gentle stream of nitrogen. In addition, using acetone for extraction gave minimum interference (Khoshnama *et al*., 2010; Takatsuki *et al*., 2003).

Processed potato and cereal based food samples usually composed of high amount of colloids and fat, which should be have de-fatting step before extraction step (Petersson *et al*., 2006). The use of defatting with n-hexane had in most cases no significant effect on the acrylamide yield, not even for fatty matrices (Pengyan *et al*., 2008) Defatting was accomplished in the same tube as the extraction.

Calibration Curve:
Standard acrylamide was determined with peak observed at 3.5. A good coefficient of correlation of 0.9977 was estimated (Figure1). The limit of LOD (signal to noise 3) 10.5 µg/kg was detected, while the limit of quantification LOQ (signal to noise 10) was 33.3 µg/kg. Laboratory experiments showed that its recovery was at level of 85% ±5%.

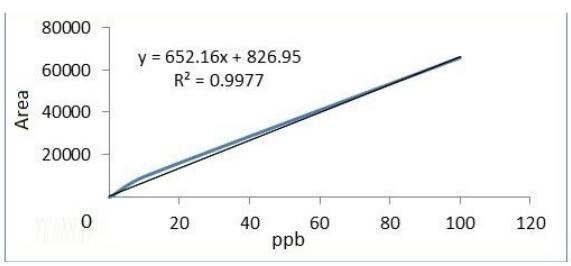

Figure 1: Calibration curve of acrylamide.

The analysis of potato chips was done, 14 characteristic peaks were observed. Figure 2 show chromatogram of potato chips spiked with acrylamide. Two corn snacks products were analyzed 11characteristic peaks observed. Figure 3 a, show corn flakes chromatogram. The same sample spiked with acrylamide at level 2000 µg/kg (Figure 3 b) . Acrylamide was eluted at 3.5 min.

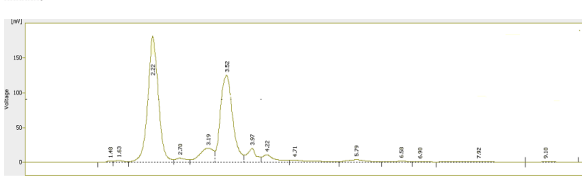

Figure 2: Potato chips chromatogram spiked with acrylamide.

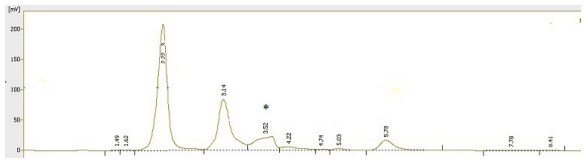

Figure 3a: Chromatogram of corn flakes, non-spiked.

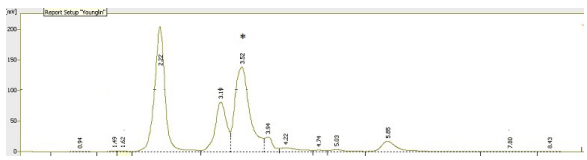

Figure 3 b: Chromatogram of corn flakes spiked with acrylamide at a level of 2000 µg/kg.

The levels of AA varied considerably between single foodstuffs within food groups (Table 1). AA levels in food and drink groups (according to the highest level in comparative samples within the same group). The potato chips group contained the highest level of acrylamide compared with the other six groups. In potato chips group, highest level of acrylamide has been found in crisp potato (1226 µg/kg) followed by potato chips 2-flavored tomato (773 µg/kg). The lowest level of acrylamide in potato chips group was found in potato chips 1-flavored salt (560 µg/kg). Level of AA in corn snacks group ranged from 553 µg/kg in corn flavored cheese to 620 µg/kg in corn flavored pepper (Table 1). In the breakfast cereals group, corn flakes had moderate level of acrylamide 354 µg/kg and 233µg/kg. Bread group had a variation in AA levels, crisp bread butter was the highest level (528 µg/kg) while Arabian white bread was the lowest level (70 µg/kg). In addition, biscuit had 170 µg/kg acrylamide. Moreover, AA level in soluble coffee was 420 µg/kg while roasted Turkish coffee had lower amount of AA (130 µg/kg). In baby food group AA level ranged from 20 µg/kg for cereal baby foods to 56 µg/kg for jarred baby foods).

The levels of AA in the data presented in Egyptian markets are comparable with previous published data in other countries (Table 1). Highest level of acrylamide has been found in potato crisp. The increasing amount of AA may be due to processing implemented by industry. Potato chips and snacks had lower amount of acrylamide. The present results are in agreement with previous survey studies on AA level (Svensson *et al*., 2003; Bekas *et al*., 2006 and Moiska *et al*., 2012). Formation of acrylamide in potato chip fried traditionally or under vacuum increase either with time or temperature (Granda and Moreira, 2005). Cereals have differing potential for the formation of AA, depending on their type and varying content of free asparagine. Raw materials with low asparagine contents cause the extrusion process to form end products with low AA values.

Acrylamide is formed in coffee beans due to Maillard reaction during roasting that takes place at high temperature, for example, in range of 220-25°C. The degree and speed of roasting affects acrylamide formation due to formation kinetics (Lantz *et al*., 2006). Roasting causes acrylamide to form in coffee beans beyond the commonly accepted asparagine/sugar condensation (Guenther *et al*., 2007).

Crisp bread had the highest level of acrylamide in bread group. While white bread contained higher AA than dark bread. However, AA formation is varied depending on flour type, ingredients, temperature time and layer thickness. Lowest concentration of AA was observed in baby food in current study. Jio *et al*. (2005) reported AA level in cereal baby food from 6.8 to 124.9. EFSA (2010) reported that the arithmetic mean acrylamide content ranged from 44 to 353 µg/Kg for 'jarred baby foods. This variation in current study and also the variation in other re-

ported studies related to acrylamide formation factors such as content of reducing sugar, free asparagine, industrial processing including temperature, pH and surface to volume ratio of food material.

However, FAO and WHO (2002) recommended that, AA presumably would be present in acceptable levels in nutritionally balanced diet levels in individual foods should be as low as reasonably achievable.

Table 1: Acrylamide level in selected foods and drinks in Egypt.

Food group	Food subgroup	AA level µg /Kg	AA level µg /Kg in previous published data
Potato chips	Chips1 flavored salt	560	468-656 (U.S. Food and Drug Administration 2003); 380-755 (Bekas et al., 2006); 14-40 (Brill et al., 2005)
	Chips1flavored tomato	646	
	Chips 2 flavored pepper	605	
	Chips 2 flavored tomato	773	
	Crisp potato	1226	693 (U.S. Food and Drug Administration, 2003)
Corn snacks	Corn flavored pepper	620	NI
	Corn flavored cheese	553	NI
Breakfast cereals	Corn flakes1	233	77 (U.S. Food and Drug Administration, 2003)
	Corn flakes2	354	
Bread	Arabian white bread	70	180 (Al-Dmoor et al.,2004); 90 (El-Ziney et al.,2009)
	Arabian dark bread	58	40 (El-Ziney et al.,2009)
	Crisp bread butter	528	427(Valentina et al.,2012)
	Crisp bread olive	196	
Biscuits	Biscuit fingers	170	75-2520 (EFSA, 2010)
Coffee	Soluble Coffee	420	240-359 (U.S. Food and Drug Administration, 2003)
	Roasted Turkish Coffee	130	351(Friedman, 2003); 259 (Svensson et al., 2003)
Powdered baby food cereal and milk	Cereal baby food 1	45	6.8-124.9 (Jio et al., 2005); 69-353 (EFSA 2010)
	Cereal baby food 2	20	
	Jarred baby food1	56	44-162 (EFSA, 2010)
	Jarred baby food2	40	

NI: No information

Conclusion:

Acrylamide is formed via the Maillard reaction between reducing sugars and asparagine in carbohydrate-rich foods during heat treatment processes such as frying, baking, roasting and extrusion. This study used HPLC methodology for the quantitative analysis of acrylamide in baby food cereals and baby food in jars. This method is successfully applied to the quantification of acrylamide in baby foods, which had low acrylamide content 20-56µg/ kg.

A survey of selected commercial foods in Egypt, foods from the Egypt market was performed for determining the AA contents in these products. The highest level of AA was determined in Potato crisps and chips. In general, snacks, cereals, crisp bread and soluble coffee were among the food products with high levels of AA. Significant differences were observed in the AA contents of different brands of the baby food, chips and cereal. Moreover, it is highly required to establish AA determination method. RP-HPLC-PDA method used as a convenient, reliable and low-cost method and can be easily adopted by non-specialized analytical laboratories. Therefore, the present study monitored the concentrations of acrylamide in selected products and baby foods at available markets in Egypt by HPLC.

REFERENCE

Al-Dmoor, H.M.; Humeid, M.A. and Alawi, M.A. (2004). Investigation of acrylamide levels in selected fried and baked foods in Jordan. Int. J. Food Agric. Environ. 2: 157-165. Bekas, W.; Kowalska, D. and Kowalski, B. (2006). Acrylamide in commercial potato chips from warsaw market Pol. J. Food Nutr. Sci. (4): 391–394. Brill, K. A.; Li, S.J.; Kwak, H.A. and Lin, J.D. (2005). Detection of acrylamide potato chips via soxhlet extraction and GC/MS analysis. Biochemistry 29-33. Brown, L.; Rhead, M.; Bancroft, K. and Alen, C. (1980). Industrial use of acrylamides. Water Pollut. Control, 79: 507–510. EFSA: European Food Safety Authority scientfic report of EFSA (2010). Results on acrylamide levels in food from monitoring year 2008. EFSA J. 8 (5):1-31. Elhassaneen, Y. and Sayed R., S. (2015). Chemical and toxicological evaluation of some snacks consumed by children in Egypt. Int J Nut Food Sci. 4(4): 493-502. El-Ziney, M.G.; Al-Turki, A.A. and Tawfik, M.S. (2009). Acrylamide status in selected traditional Saudi foods and infant milk and foods with estimation of daily exposure. Am J Food Technol. 4: 177-191. Eriksson, S. (2005). Acrylamide in food products: Identification, formation and analytical methods. PhD., Stockholm university. Pp 91. Faca, V.; Coram, M.; Phanstiel, D.; Glukhova, V.; Zhang, Q.; Fitzgibbon, M. and Mclntosh, S. (2006). Quantitative analysis of acrylamide labeled serum proteins by LC-MS/Ms. Proteomeres, 5, 2009- 2018. FAO and WHO (Food and Agriculture Organization of the United Nations in collaboration with the World Health Organization) (2002). Consultation of health implications of acrylamide in food.Summary report of meeting held in Geneva, June 25-27. Available at www.fda.gov/ohrms/dockets/dailys/02/.../02n-0393-rpt0001-vol1.pdf. Friedman, M. (2003). Chemistry, biochemistry and safety of acrylamide: A review. J. Agric. Food Chem. 51: 4504-4526. Granda, C. and Moreira, R.G. (2005). Kinetics of acrylamide formation during traditional and vacuum fraying of potato chips. J. food process eng. Vol28 issue 5: 478-493. Guenther, H.; Anklam, E. and Wenzl, T. (2007). Acrylamide in coffee: review of progress in analysis, formation and level reduction. Food addit contam. 2460-2470. Hoenicke, K.; Gatermann, R.; Harder, W. and Hartig, L. (2004). Analysis of acrylamide in different foodstuffs using liquid chromatography-tandem mass spectrometry and gas chromatography-tandem mass spectrometry. Anal Chem. Acta, 520: 207-216. IARC (International Agency for Research on Cancer) (1994). IARC monographs on the evaluation of carcinogenic risks to humans: some industrial chemicals. Lyon, France: IARC. 60, 389–433. Jio, J.; Zhang, Y.; Yiping, R.; Wu, X. and Zhang, Y. (2005). Development of a quantitative method for determination of acrylamide in infant powdered milk and baby foods in jars using isotope dilution liquid chromatography/electrospray ionization tandem mass spectrometry. J. Chromatography A.1099: 198-202. Khoshnama, F.; Zargarb, B.; Pourrezab, N. and Parhamb, H. (2010). Acetone extraction and HPLC determination of acrylamide in potato chips. J Iran. Chem. Soc. 7(4): 853-858. Kopp, E.K. and Dekant, W. (2009). Toxicokinetics of acrylamide in rats and humans following single oral administration of low doses. Toxicol. Appl. Pharmacol. 235: 135–142. Lantz, I.; Ternite, R. and Wilkens, J. (2006). Studies on acrylamide levels in roasting, storage and brewing of coffee. Mol nutr food res. 50 (11) 1039-1046. Matuszewski, B.K.; Constanzer, M.L. and Chavez-Eng, C.M. (2003). Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC. Anal. Chem. 75: 3019-3030. Michalak, J.; Gujska, E. and Kuncewicz, J. (2013). RP-HPLC-DAD studies on acrylamide in cereal-based baby foods. J Food Composition and Anal. 32 (1): 68–73. Moiska, H.; Gieleclnska, I. and Stos, K. (2012). Determination of acrylamide level in commercial baby foods and assessment of infant dietry exposure. Food Chem toxical. 50 (8): 2722-2728. Pengyan, L.; Li, Z. and Lei, L. (2008). Determination of acrylamide in potato chips by high-performance liquid chromatography coupled to diode array detection. Chemical J Inter. 10 (2) P9 .http://www.chemistrymag.org/cji/2008/102009pe.htm. Petersson, E.V.; Rosen, J.; Tuner, C.; Danielsson, R. and Hellenas, K.E. (2006). Critical factors and pitfalls affecting the extraction of acrylamide from foods, an optimization study. Anal chem Acta, 557, 287-95. Saleh, S.I. and El-Okazy, A. M. (2007). Assessment of the mean daily dietary intake of acrylamide in Aexandria. J Egypt Public Health Assoc. 82(3-4): 331-345. Singh1, P. and Balaji, R. (2010). Determination of acrylamide concentration in processed food products using normal phase high performance liquid chromatography (HPLC). African J Biotech. 9(47): 8085-8091. Svensson, K.; Abramsson, L.; Becker, W.; Glynn, A.; Hellanas, K.E.; Lind, Y. and Rosen, J. (2003). Dietary intake of acrylamide in Sweden. Food Chem. Toxicol. 41: 1581-1586. Takatsuki, S.; Nemoto, S.; Sasaki, K. and Maitani, T. (2003). Determination of acrylamide in processed foods by LC/MS using column switching. Shokuhin Eiseigaku Zasshi. 44(2):89-95 http://www.ncbi.nlm.nih.gov/pubmed/12846155. U.S. Food and Drug Administration. (2003). Exploratory Data on Acrylamide in Foods. Available at https://cspinet.org/new/pdf/acrylamide_product_charts.pdf. Valentina, L.; Christova-Bagdassarian, J. Tishkova, A. and Terry, M. V. (2012). Acrylamide in Processed Foods. Bulgarian J Chem. 1 (3): 123-132. Wenzl, T.; Beatriz De La Calle, M. and Anklam, E. (2003). Analytical methods for the determination of acrylamide in food products: a review, Food Addit Contam: Part A, 20: 885-902.