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Acrylamide Levels in Selected Foods in Saudi Arabia with Reference to Health-Risk Assessment of Dietary Acrylamide Intake

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Abstract: The acrylamide (AA) levels in marketing foods in gulf area are not investigated yet. An LC-MS/MS method for the determination of acrylamide in some selected food (local/imported) has been described. The samples were pre-dried, crushed/minced, degreased and mixed with [D_3] acrylamide internal standard then acrylamide was water extracted at 60°C in a ultrasonic bath. The aqueous solution was clean-up using a Carrez-Precipitation followed by centrifugation. The clean-up extract was then analyzed by LC-MS/MS. The method was applicable to detect acrylamide in different food types. The detection limit was as low as 30 μg kg^{-1}. The AA level in different food groups were in order, mashed-roasted potato > fried pasta > soluble coffee > biscuits > potato chips > cocoa powder > crisp bread > fired rice > roasted Turkish coffee > cereal breakfast (corn) > butter cookies. The highest value of acrylamide (8974 μg kg^{-1}) was detected in mashed-roasted potato, whereas the lowest value was detected in butter cookies (151 μg kg^{-1}). The calculated average daily intake amounted to 34.03 μg AA/person/day which corresponds to 0.57 μg kg^{-1} body weight/day (body weight 60 kg). The outcome of this study has strongly recommended the necessity to conduct a large-scale survey in order to evaluate the levels of acrylamide in traditional foods. Thus, the true risk levels related to AA intake will be accurately estimated.

Key words: Acrylamide, food, coffee, dietary daily intake

INTRODUCTION

In April 2002, both Swedish National Food Administration (SNFA, 2002) and University of Stockholm announced that certain cooked processed foods at relatively high temperatures contain significant levels of acrylamide.

Acrylamide (2-propenamide) is a highly water-soluble (2144 g L^{-1}) compound with a low molecular weight (71.09). It is a reactive chemical, which is used as monomer in the synthesis of polyacrylamides used e.g., in purification of water, conditioning of soil and separation of proteins in analytical biochemistry and it is known as a component in tobacco smoke (Allan, 2002; Grives *et al*., 2002). Acrylamide has been classified as a group 2A carcinogen (probably carcinogenic to human) by the International Agency for Research on Cancer (1994). This might represent a potential threat to public health (FAO/WHO, 2002, 2005). Animal studies documented that high doses of acrylamide (>203 mg kg^{-1}) caused adverse developmental and reproductive effects in neonatal rodents (Friedman, 2003). For example, nerve degeneration, deficiency of intestinal enzymes, abnormal sperm and reduced

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fertility have been observed in treated rodents. On the other hand, in epidemiological studies for different types of cancer, it has not been possible to fined positive evidence for relationship between dietary acrylamide and cancer (Wirfalt *et al*., 2008). Neither in hospital-based control studies (Pelucchi *et al*., 2005) nor in epidemiological studies for large bowel, kidney and bladder cancer (Mucci *et al*., 2003) were observed. However, it is concluded that the statistical power of standard epidemiological studies is too low to detect an increased risk for cancer due to background exposure to acrylamide (Hagmar *et al*., 2005).

In view of this, the FAO/WHO consultation acknowledged the potential link between acrylamide and carbohydrate-rich food cooked excessively, for too long time or/and at very high temperature. The consultation considered that existing data is insufficient, especially outside Europe and North America to allow a quantitative estimate of health risks from dietary acrylamide and urged for more research on acrylamide in food in this area (FAO/WHO, 2002, 2005).

Arabian traditional carbohydrate-rich foods are an all-time favorite in Arab countries. These including a wide range of products, such as fried rice and pasta (Shaerria), Conaffa, Om Alli etc. Where such food exposed to high-temperature cooking e.g., roasting, backing, frying, grilling, which have the same manner of preparation with lots of western foods. Therefore, Arabian traditional carbohydrate-rich foods may represent high-risk levels of acrylamide under heating processes. To our best knowledge, there is a little information reported on the level of acrylamide in Arabian area.

The objectives of this study were to determine the levels of acrylamide in selected home-made and marketing foodstuffs known for its high concentration of acrylamide in Saudi market, to evaluate the effect of some traditional cooking processes on the level of acrylamide and further, risk assessment of dietary acrylamide.

MATERIALS AND METHODS

Chemicals

Acrylamide (99%) was from Sigma (MO, USA). [D_3] acrylamide internal standard ISTD (89%) was obtained from CIL (Andover, MA, USA). Potassium hexacyanoferrate (3 H_2O), Zinc sulphate ($7H_2O$), sodium chloride, sodium sulphate anhydrous and acetic acid were analytical grade from Merck (Darmstadt, German). Acetic acid ethylester, methanol (durability: 3 months), iso-hexane, acetonitrile (min 99%) were HPLC grade obtained from Merck (Darmstadt, German). Deionised water was purified using a water purification system (Millipore, Mohlsheim, France).

Food Samples

Thirty-two samples of various foodstuffs (9 groups) were analyzed in this study. The selected food groups were potato (mashed roasted and chips), crisp bread, biscuit (Steak), cookies (Butter cookies), rice (fried), pasta (Shaerria; like nodal), breakfast cereals, cacao and coffee (soluble and roasted Turkish). Out of total samples, ten were collected from the local stores. Half of it was locally produced while the other half was imported. The last two samples; fried rice and pasta, were fried in the Laboratory to simulate the cooking procedure of making rice in the Arabian kitchen. A portion of soaked rice and dried fine pasta were deep-fried in fresh vegetable oil at 170±10°C for 10 min until it became substantial browning.

Acrylamide Analysis

In general, the given sample was pre-dried, crushed/minced, degreased and mixed with deuterated internal standard [D_3] then acrylamide was water extracted at 60°C in a ultrasonic bath. The aqueous solution was clean-up using a Carrez-Precipitation followed by centrifugation. The clean-up extract was then analysed by LC-MS/MS. In the case of complex matrices, clean-up extract was directly re-extracted with ethylacetate and acrylamide in organic phase was analysed by LC-MS/MS. The quantification was done against the Internal Standard (Hoenicke, 2003).

Preparation of Sample

Damp groceries were minced in a meat grinder (Jupiter) and made homogeneous. Dry groceries were crushed in a Retsch-mill and made homogeneous. Bread and fine cakes and pastries have to be pre-dried carefully using Turbovap 500 concentrator. The sample material was mixed well and then filled into a sample cup.

Processing of Sample

Depending on the groceries' matrix a certain processing was chosen. In the case of dry groceries containing starch (cornflakes, potato chips, crisp-bread) and roasted coffee, two grams of the fine-grained or pulverized sample was weighed in on a filter paper. The filter was directly set on an aspiration apparatus to degrease the sample by arranging a slight vacuum with altogether 80 mL iso-hexan. The residue was then quantified and transferred into a 150 mL beaker. After that it mixed with a 200 μL ISTD-solution (c = 10 μg mL^{-1}) and kept still for 30 min. Then a portion of 20 mL of water was added and the sample suspension was extracted at 60°C in an ultrasonic bath (Bandeline Sonderex Super RK 510 H, Germany) for 30 min. Next 20 mL acetonitrile and each of 500 μL Carrez I (150 g of potassium hexacyanoferrate/l) and Carrez II (300 g of zinc sulphate/l) were adjoined, whereby the solution has to be well-stirred after each adding up. Then, the aliquot was centrifuged (Hettich EBA 85, Germany) at 4500 g for 10 min. The resulted supernatant was membrane-filtered (Acrodisc 0.45 μm, Pall Gelman Lab., USA) in an autosampler-vial.

In the case of samples containing high amount of fat (fats for frying), 40 mL iso-hexan were brought out on a 150 mL partition funnel. Two grams of sample were weighed in the partition funnel and finally dissolved. A portion of 20 mL water and 200 μL ISTD-solution (10 μg mL^{-1}) were then added and sturdily shaken. After that 10 mL of the aqueous phase were transferred into a 150 mL beaker and then mixed with 10 mL acetonitrile and each 500 μL Carrez I and Carrez II, whereby the solution has to be well-mixed after each adding up. Then centrifugation and filtration were followed as mentioned above.

In the case of Cacoa powder, 2 g of the fine-grained sample were weighed then mixed with 50 mL water as well as with 200 μL ISTD-solution (c = 10 μg mL^{-1}). After that it was extracted in an ultrasonic bath at 60°C for 30 min. The extracted was transferred into a 250 mL centrifuge cup and mixed with 30 mL iso-hexan as well as with each 500 μL Carrez I and Carrez II. After each addition of a substance the solution was well-shaken. Then, it was centrifuged for 10 min at 4500 g. The aqueous phase was taken up and transferred into a 150 mL partition funnel where was mixed with sodium chloride until the saturation point then it was well-shaken. The aqueous phase was shake out two times with 50 mL ethylacetate. The united organic phases were given over a filter (filled with sodium sulphate) in a turbo-wap-container, narrowed down and then membrane-filtered in an autosampler-vial.

Identification and Quantification

LC-MS/MS analyses were performed using Sciex® API 2000 mass spectrometer system coupled with an Agilent 1100 HPLC equipped with tempered well plate autosampler, a pump and a pillar stove (Applied Biosystemes, Darmstadt, Germany).

For HPLC analysis of the extracts, 40 μL were injected onto LiChro CART 250-4 LiChrospher®100CN (5 μm) preceded by LiChro CART 4-4 LiChrospher®100 RP-pre-pillar-cartridges (5 μm) (Merck, KGaA, Darmstadt, Germany).

The columns were eluted with a mobile phase composed of A: acetonitrile/acetic acid, 1% (50/50 v/v) and B: acetonitrile, run at 0-5 min 100% A, 6-9 min 100% B and 10-20 min 100% A. The flow rate was 0.7 μL min^{-1} and the MS/MS transitions (m/z) monitored for acrylamide were 72 and 55, those for [D_3] acrylamide (the internal standard) were 75 and 58. The quantification and calibration was based on the 72>55 and 75>58 mass passages/ mass transitions. The calculation equation was as follow:

$$\text{Acrylamide } (\mu g\ kg^{-1}) = \frac{\text{Area of AA} \times 2 \times 1000}{\text{Area of } [D_3]\text{AA} \times \text{EW}}$$

where, Area AA is a peak-area at 72>55 transitions, Area $[D_3]$ AA is a peak-area at 75>58 transitions and EW is a specimen net weight (g).

Additionally this analysis was integrated within the scope of official accredited and validated analyses in the Laboratory of Food Analysis.

Analytical Quality Assurance

Analytical quality assurance measures were employed for acrylamide, which involved inclusion in duplicate of 2 g of rolled oats spiked at 5 μg kg^{-1} (ppb) and reagent blank. The same series of samples was also spiked with labeled acrylamide. Batches of samples were deemed acceptable if spiked samples (with labeled acrylamide) indicated >80% recovery rate.

Food Consumption Data and AA Intake

Food consumption data were based on a quantitative frequency questionnaire answered by 100 female subjects aged 18-25 years who recorded their food consumption during a week. All estimated intakes were adjusted for the individual's self-reported body weight (bw = 60 kg; WHO, 1983) and expressed as daily. AA daily intake calculated according to the following model:

$$\text{AA Intake} = (\text{Eaters (y/n)}) \times (\text{Food amount}) \times (\text{AA level})$$

Eaters (yes or no), either 0 or 1 in proportion to percent eaters; Food amount, food consumption value (g) from survey data; AA level, acrylamide value from laboratory analysis (μg kg^{-1}). Each data value equally likely on each iteration. Results are summed over foods and individuals.

RESULTS AND DISCUSSION

The calibration curve was computed using the area ratio of the acrylamide (72-55 m/z) peak to that of the D_3-acrylamide (75-58 m/z). The correlation coefficient was $R^2 = 0.9991$ (Fig. 1) and the detection limit was found to be 10 μ kg^{-1} while the quantification limit was set at 30 μ kg^{-1}. The recovery was in the range of 80-100% with reproducibility is higher than 5%. A sample chromatogram is given in Fig. 2, which shows an extract of potato chips with AA level up to 2100 μg kg^{-1}.

In this study, food samples from nine food groups (11 sub-groups) were analyzed for their AA contents. It is important to note that present results cannot provide guidance in a consumer's choice

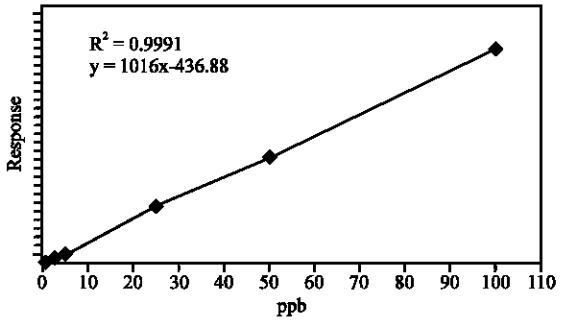

Fig. 1: Calibration curve of Acrylamide

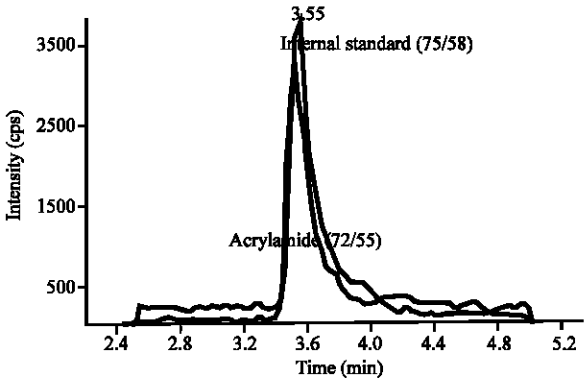

Fig. 2: LC/MS-MS chromatogram of potato chips containing 2100 μg kg^{-1} acrylamide and 2000 μg kg^{-1} [D_3]-labelled acrylamide

Table 1: Acrylamide (AA) levels in selected food and drink groups in Saudi market compared with published survey data

		AA level (μg kg^{-1})	
Food group	Sub-group/State	Present study	Published data
Potato	Mashed roasted	8974	NI*
	Chips	620	170-3700[1]; 693-2150[2]; 550[5]; 433[7]
Crisp	Bread	439	800-1200[1]; 270-740[4]; 153[6]; 300[7]
Cereal	Breakfast	215	30-1346[1]; 11-89[2]; 220[3]; 100[4]; 105[5]; 95[6];112[7,8]
Pasta	Fried	1600	NI
Rice	Fried	430	67[4]; 386[9]
Biscuit	Steak	810	30-3200[1]; 300[3]; 389[7]
Cookies	Butter cookies	151	70-430[1]; 300[3]; 71-240[4]; 204[5]; 275[6]
Cocoa	Powder	256	280[5]
Coffee	Soluble	816	37-374[2], 3-13[4]; 270[5]; 204[6]
	Roasted Turkish	282	170-351[1]; 25[3]

NI: No Information; [1]Friedman (2003), [2]USFDA (2008), [3]Svensson *et al.* (2003), [4]Leung *et al.* (2003), [5]Hoenicke *et al.* (2004), [6]Murkovic (2004), [7]Şenyuva and Gökmen (2005), [8]Claus *et al.* (2008) and [9]Zhang *et al.* (2007)

between food products and brands; however, it could be used as a general guide to assess the AA levels in a selected segment of the Saudi food supply. The levels of AA varied considerably between single foodstuffs within food groups (Table 1). Regarding the mean AA levels, food and drink groups were in the order mashed roasted potato (8974 μg kg^{-1}) > fried pasta; Shaerria (1600 μg kg^{-1}) > soluble coffee (816 μg kg^{-1}) > biscuit (810 μg kg^{-1}) > potato chips (620 μg kg^{-1}) > cocoa (526 μg kg^{-1}) > crispbread (439 μg kg^{-1}) > fried rice (430 μg kg^{-1}) > roasted Turkish coffee (282 μg kg^{-1}) > breakfast cereal (215 μg kg^{-1}) > butter cookies (151 μg kg^{-1}). It is postulated that higher AA concentrations up to above 1000 μg kg^{-1} were determined in the two food groups roasted potato and Shaerria, where the lowest concentration was observed in the cookies butter group. Moreover, soluble coffee contained a higher amount of AA compared with roasted Turkish one. Arabian traditional carbohydrate-rich foods may represent consumer concern since, deep frying of rice and pasta raised AA level considerably as compared to raw materials (<30 μg kg^{-1}).

It is postulated that the present results are in comparable with published data however, it showed a wide range of AA for the same group of food (Table 1). This high variability among the survey data is mainly resulted from the variable heating production processes (°C/time) and variable composition of raw materials. The coffee had the highest contribution to our AA dietary intake, especially the soluble type compared to Turkish roasted coffee meanwhile; steak biscuits had the same high

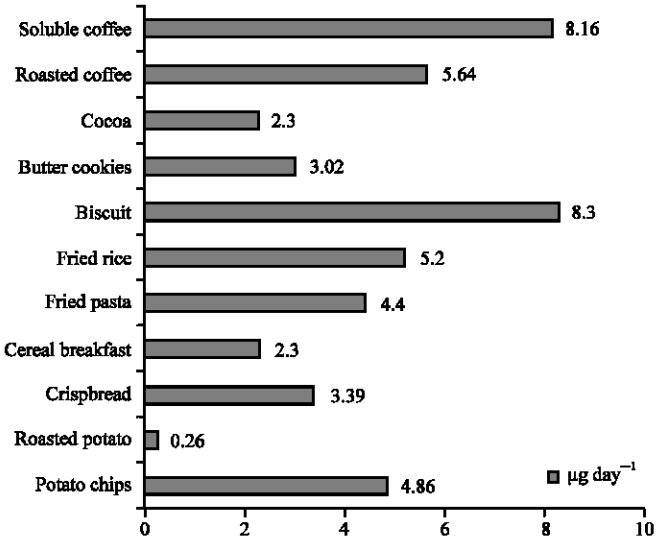

Fig. 3: Dietary intake of Acrylamide from each analyzed food groups

contribution to AA dietary intake (Fig. 3). The way of cooking could represent a health risk factor such as the case of fried pasta and rice further, present study confirmed the input of potato chips towards AA intake (Fig. 3).

The average estimated daily intake amounted to 34.03 µg AA/person/day which amounted to 40 µg/p/d in the case of adding coffee to the daily diet. This is corresponding to 0.57 µg AA/kg body weight/day (body weight of 70 kg).

The FAO/WHO assumes that the range of dietary intake of AA has a range of 0.3-0.8 µg kg^{-1} body weight/day in the developing countries.

It is concluded from the present study that the levels of acrylamide in different food groups are great varied which could be reflected the differences in food composition (e.g., nature of carbohydrate; free amino acid content) and heat treatment (e.g., temperature, time, type of heat convection). Hence, it is strongly recommended that large-scale research studies regarding the levels of acrylamide in traditional and marketing Gulf foods should be conducted to give a validating evaluation about acrylamide dietary intake. Further, look for innovating a technological processes able to reduce it. Meanwhile, for nutritional and preventive consideration it is suggested that risk groups (children and adolescents) should eat a balanced and varied diet. This includes high amount of vegetables and fruit with moderate consumption of cereals, fried rich-carbohydrate foods and coffee. Hence, AA presumably would be present in acceptable levels in nutritionally balanced diet however; levels in individual foods should be as low as reasonably achievable (FAO/WHO, 2002).

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REFERENCES

Allan, S., 2002. Acrylamide angst; another annoying distraction about food safety. (Electronic version). Agrochemical and Environmental News Issue, October, No. 198. available at http://www.envirofacs.org/Acrylamide%20Anst.pdf.

Claus, A., R. Carle and A. Schieber, 2008. Acrylamide in cereal products: A review. J. Cereal Sci., 47: 118-133.

FAO/WHO, 2002. Consultation of health implications of Acrylamide in food. Summary report of meeting held in Geneva, 25-27 June 2002, WHO (World Health Organization).

FAO/WHO, 2005. Evaluation the health risks of several chemical contaminants in food. Report of a Joint FAO/WHO expert committee on food additives (JECFA). 9-17 February 2002, WHO (World Health Organization).

Friedman, M., 2003. Chemistry, biochemistry and safety of acrylamide. J. Agric. Food Chem., 51: 4504-4526.

Grives, S., M. Jägersted, H. Lingnert, K. Skog, M. Törnquist and P. Åman, 2002. Acrylamide in food-Mechanisms of formation and influencing factors during heating of foods. http://www.li.se/document/news/nyheter/Acrylamide%20Expertrepot%20final.pdf.

Hagmar, L., E. Wirfält, B. Paulsson and M. Törnqvist, 2005. Differences in hemoglobin adduct levels of acrylamide in general population with to dietary intake, smoking habits and gender. Mutat. Res., 580: 157-165.

Hoenicke, T, 2003. Determination of acrylamide in groceries (institution's/company's method), Applied Biosystems, Replacement: PRU 0064-1, Parameter No: W 1860/W 1852.

Hoenicke, K., R. Gatermann, W. Harder and L. Hartig, 2004. Analysis of acrylamide in different foodstuffs using liquid chromatography–tandem mass spectrometry and gas chromatography-tandem mass spectrometry. Analytical Chimica Acta, 520: 157-165.

International Agency of Research on Cancer (IARC), 1994. Acrylamide, IRAC Monographs on the evaluation of the carcinogenic risk of the chemicals to humans, vol. 60, International Agency of Research on Cancer, Lyon.

Leung, K.S., A. Lin, C.K. Tsang and S.T.K. Yeung, 2003. Acrylamide in Asian foods in Hong Kong. Food Additives Contam., 20: 1105-1113.

Mucci, L. A., P. W. Dickman, G. Steineck, H.O. Adami and K. Augustsson, 2003. Dietary acrylamide and cancer of the bowel, kidney and bladder: Absence of an association in a population-based study in Sweden. Br. J. Cancer, 88: 84-89.

Murkovic, M., 2004. Acrylamide in Austrian foods. J. Biochem. Biophys. Methods, 61: 161-167.

Pelucchi, C., C. Galeone, F. Levi, E. Negri, S. Franceshi, R. Talamini, C. Bosetti, A. Giacosa and C. La Vecchia, 2005. Dietary acrylamide and human cancer. Int. J. Cancer, 118: 467-471.

Şenyuva, H.Z. and V. Gökmen, 2005. Survey of acrylamide in Turkish foods by an in-house validated LC-MS method. Food Additives Contam., 22: 204-209.

SNFA (Swedish National Food Administration), 2002. Information about acrylamide in food. http://www.slv.se.

Svensson, K., L. Abramsson, W. Becker, A. Glynn, K.E. Hellanäs, Y. Lind and J. Rosçn, 2003. Dietary intake of acrylamide in Sweden Food Chem. Toxicol., 41: 1581-1586..

USFDA, 2008. Exploratory data on acrylamide in food. CFSAN, www.cfsan.fda.gov/~dms/acrydata.html.

WHO, 1983. Measuring change in nutritional status: guideline for assessing the nutritional impact of supplementary feeding program Geneva: World Health Organization, pp: 86-97.

Wirfalt, E., B. Paulsson and M. Törnqvist, 2008. Associations between estimated acrylamide intakes, and hemoglobin AA adducts in a sample from the Malmo Diet and Cancer cohort. Eur. J. Clin. Nutr., 62: 314-323.

Zhang, Yu, Y. Ren, H. Zhao and Y. Zhang, 2007. Determination of acrylamide in Chinese traditional carbohydrate-rich foods using gas chromatography with micro-electron capture detector and isotope dilution liquid chromatography combined with electrospray ionization tandem mass spectrometry. Analytical Chimica Acta, 584: 322-323.