Acrylamide Status in Selected Traditional Saudi Foods and Infant Milk and Foods with Estimation of Daily Exposure

[1]M.G. El-Ziney, [2]A.A. Al-Turki and [1]M.S. Tawfik
[1]Departments of Food and Dairy Sciences,
Faculty of Agriculture, Alexandria University, Al Shatby, Egypt
[2]Department of Plant Production and Protection,
College of Agriculture and Veterinary Medicine, Qassim University,
P.O. Box 2266, Buraidah 51452, Saudi Arabia

Abstract: This study reports the results of the survey study on acrylamide levels in selected traditional foods and infant powder milk and cereal based foods obtained from the Saudi market. Food samples divided into twelve groups. An LC-MS/MS method for the determination of acrylamide (AA) 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 $60°C$. 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 AA in different food types at concentration of $\cong 30$ µg kg^{-1}. The extraction method was developed to enable detecting of traces of AA. A second sensitive extraction method was followed in order to allow a concentration of AA as low as 1-5 µg kg^{-1}. In general, the acrylamide (AA) level in different food groups were in order, grilled egg-plant>coffee (soluble)>extruded maize>cookies (korse Omer; tweel) and biscuit>extruded maize (cheese) and cookies> French fries>sweet (zalabia)>bread and cooked palm date (Hunaini)>out layer of fried fish>infant powder milk and cereal foods. The highest value of acrylamide (950 µg kg^{-1}) was detected in grilled egg-plant whereas the lowest value was detected in baby powder milk (3.4 µg kg^{-1}). The calculated daily intake amounted to 60 µg AA/person/day which corresponds to 0.86 µg kg^{-1} b.wt. day^{-1} (body weight of 70 kg). The average daily AA dietary intake of different infant milk brands, analyzed in the present study, during the first six months of birth amounted to 0.63 µg day^{-1}. This is corresponding to 0.075 µg AA kg^{-1} b.wt. day^{-1} (body weight of 8 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, traditional food, coffee, infant milk, baby foods, dietary daily intake

INTRODUCTION

Acrylamide (CH2 CHCONH2, CAS Registry Number 79-06-1) is an important industrial monomer, and has been available commercially since the mid-1950s. Acrylamide is

Corresponding Author: Dr. Mohamed G. El-Ziney, Lab of Food Microbiology and Toxicology, Qassim University, Saudi Arabia

manufactured on a large scale (50,000-77,000 tons annually in Japan, the USA, and Europe in the early 1990s). Acrylamide (2-propenamide) is a highly water-soluble (2144 g L^{-1}) compound with a low molecular weight (71.09). Acrylamide (AA) 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).

The recent discovery that acrylamide is formed in significant quantities during high-temperature cooking of animal feed (Tareke *et al.*, 2000) and that it is measurable at significant concentrations in many common human foods that are prepared by cooking at high temperatures (Svensson *et al.*, 2003), especially frying, baking or drying, introduced a new and unexpected dimension to carcinogenic risk assessment for acrylamide, one that is still evolving. It has been shown that acrylamide is formed during the cooking of foods principally by the Maillard reaction between the amino acid asparagine and reducing sugars such as glucose (Mottram *et al.*, 2002; Stadler *et al.*, 2002). Since acrylamide has been classified as a Group 2A carcinogen by the International Agency for Research on Cancer (IARC, 1994) and a Category 2 carcinogen and Category 2 mutagen by the European Union (http://ecb. jrc.it/classification-labelling/), this finding caused worldwide concern (WHO, 2002).

This might represent a potential threat to public health (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 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. Neither in hospital-based control studies (Pelucchi *et al.*, 2006) 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). Validation data were presented for products from potatoes and cereals in a range from 30-10 000 µg kg^{-1}. Since, then several analytical procedures for AA in food have been developed and published, the majority of them are GC-MS (gas chromatography mass spectrometry) or LC-MS-MS methods (Wenzel *et al.*, 2003; Taeymans *et al.*, 2004).

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 (WHO, 2002, 2005).

Arabian traditional carbohydrate-rich foods are an all-time favorite in Arab countries. These including a special wide range of traditional products, such as Korsan (bread group), fried rice and pasta (Shaerria), Kors Omer, Barazek and Kolijah (cookies group) Konaffa, Zalabia and Balah Alsham (sweet group) Hunaini (date palm group) 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. Infant powder milk and baby dried foods represent a major indispensable nutrient source for babies and infants. A high consumption of these foods by infant group might be a high significant source of daily exposure to acrylamide (Jio *et al.*, 2005). Hence, the

determination of acrylamide level in such foods is highly important in order to improve and control manufacturing process and evaluate the risk of consumption.

The mean intake of AA via food has been documented; in Sweden is approximately 35 µg per person and day, corresponding to 0.5 µg AA kg^{-1} b.wt. (assuming a body weight of 70 kg) (Svensson *et al.*, 2003), in Netherlands, the calculated mean intake in the Dutch population is 0.48 µg AA kg^{-1} b.wt. (Konings *et al.*, 2003) and in Norway, 0.47 µg AA kg^{-1} b.wt. (Dybing and Sanner, 2003). Concerning AA levels in baby food, few data are available. However, in one unpublished report, (www.mattilsynet.no), written by the Scientific Committee of the Norwegian Food Control Authority, based on four pooled porridge products, small children's exposure to AA was calculated. Even the smallest children, who are still being breast-fed, are exposed to AA via food. Sörgel *et al.* (2002) have demonstrated that AA penetrates into the breast milk. Since the demand for toxin-free food is extra high for children, it is important to study the AA level in baby food, including infant's milk, and estimate the AA intake. For this purpose, lower analytical detection limits are needed than for adult foods.

It is not applicable to extrapolate results of dietary exposure studies from a country to another because both eating behavior and manufacturing practices vary significantly across countries and regions (HMSO, 1993). To our best knowledge, there is a little information reported on the level of acrylamide in Arabian area (Tawfik and El-Ziney, 2008).

The aim objectives of this study were to determine the levels of acrylamide in selected traditional Saudi foodstuffs and infant powder milk and cereal foods. In this study the an improved analytical method is used. Results of AA analysis in different foodstuffs and baby foods were applied to estimate the AA intake from these products.

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 hexacyanoferrat (3 H$_2$O), Zinc sulphate (7H$_2$O), sodium chloride, sodium sulphate anhydrous, and acetic acid were analytical grade from Merck (Darmstadt, German). Acetic acid ethylester, methanol (durability: 3 months), iso-hexan, acetonitril (min. 99%) were HPLC grade obtained from Merck (Darmstadt, Germany). Deionised water was purified using a water purification system (Millipore, Mohlsheim, France).

Food Samples

This research project was done in the period of 2007-2008 in the lab of Food Microbiology and Toxicology, Qassim Universty. Twenty six of various foodstuffs included wide range of traditional foods, divided into 12 groups, were analyzed in this study. Group 1: Potato (French fries). Gruop2: Maize, extruded (cheese) and extruded (salt). Group 3: Egg plant, grilled for 15 min on direct flame until the out layer become brown, mixed with olive oil, garlic and Limon, dressing salad product known as Papa Ghanug. Group 4: Bread (a) Arabian white bread, flat, thickness 8-10 mm, pock forming, produced from white wheat flour (zero type extraction rate 72%), leavened by yeast fermentation, relatively short baking time (60-90 s at 300-350°C). (b) Arabian dark breed, produced as previous item but it is brown since whole flour is used. (c) Korsan, round solid thin layer, produced by kneading of whole flour in water then it backed on very hot surface. (d) Shabora, long rode cracks with sesame produced from whole flour leavened by yeast fermentation in addition to sodium bicarbonate, formed, backing time (10 min at 180°C). Group 5: Biscuit (a) Sugar Biscuit. Group 6: Cookies, generally a cracker type of bakery

products with substantial brown crust, composed of white flour (zero type extraction rate 72%), milk, sugar, oil, fat, water and leavened by ammonium bicarbonate, long baking time (20 min. at 280-300°C) included (a) Honey Fetet, (b) Korse Omar, (c) Barazek and (d) Teweel. (e) Kolijah and (f) Kolijah Haal were stuffed with sugar, cinnamon and ginger while (g) Mamul stuffed with palm date. Group 7: Fish (a) Grilled out layer which dipped in whole flour. (b) Fried out layer which dipped in whole flour. Group8: Sweets (a) Konaffa: A crust made of vermicelli like threads prepared from a white flour batter, baked in a metal plate on flame heater after dying and adding shortening and filling with desalted white cheese, sweeten after baking with a heavy sugar syrup, baked at 180-200°C/contact surface for ~10 min., analysis was carried out on the crust without filling, crust was 40% of the whole sweet. (b) Zalabia and (c) Balah Alsham: fried dough resembling doughnuts type sweet, substantial browning crust, deep-fried at 160-180°C for 6-8 min. in vegetable oil, composed of flour, milk, eggs and leavened by yeast, sweeten by dipping after frying in heavy sugar syrup. (d) Backlawa: thin layer of fermented dough stuffed with nuts and rolled backed for 10 min at 150°C then dipped in sugar syrup. Group 9: Date Palm: (a) Hunaini, by cooking a mix of Korsan and macerated date palm in vegetable oil or milk butter. Group 10: Drink group (a) Soluble Coffee (Robusta), (b) Roasted Turkish Coffee (Arabica), and (c) Barely coffee. Group 11: (a) Infant bay milk formula, included different brands which intended to use in ages just after birth until 12 months or after 6 month of birth. Group 12: Powdered baby food, were basically composed of cereal and milk in addition to fruit or honey.

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 analyzed 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 analyzed by LC-MS/MS. The quantification was done against the Internal Standard (Hoenicke, 2003).

Regression analysis and derived fitted models for standard curves were performed using SPSS 10 (SPSS, Chicago, IL).

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 (Zyrmark, Idstein, Germany). The sample material was mixed well and then filled into a sample cup.

Processing of Sample and Routine Extraction Method

Depending on the groceries' matrix a certain processing was chosen. In the case of dry groceries containing starch 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 spample suspension was extracted at 60°C in an ultrasonic bath (Bandeline Sonderex Super RK 510 H, Germany) for 30 min. Next 20 mL acetonitril and each of 500 µL Carrez I (150 g of potassium hexacyanoferrat L^{-1}) and Carrez II (c = 300 g of zinc

sulphate L^{-1}) 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 spample were weighed in the partition unnel 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 acetonitril 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 earlier.

Alternative Extraction Method for Powder Milk and Baby Foods

A potion of 50 mL of water and 200 µL of internal standard (D_3-acrylamide, 10 µg mL^{-1}), was added to 2 g of the homogenized sample. For baby food 10 g of homogenized sample plus 1 mL of internal standard (D_3-acrylamide, 10 µg mL^{-1}), was used. After extraction of acrylamide in a ultrasonic bath at 60°C for 30 min, a volume of 30 mL of iso-hexane, 5 mL of Carrez I (potassium hexacyanoferrate, c = 150 g L^{-1}) and Carrez II (zinc sulphate, c = 300 g L^{-1}) were added. The mixer was centrifuged at 4500 rpm for 10 min. The aqueous phase was saturated with sodium chloride and extracted twice with 50 mL of ethyl acetate. The combined organic phases were concentrated to 1 mL using a Turbo Vap 500 (Hoenicke *et al.*, 2004).

Identification and Quantification of AA

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 analyses 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:

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

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

The daily uptake of AA through the consumption of some food groups analyzed in this study was estimated. Food consumption data were based on a quantitative frequency questionnaire answered by 50 male/female subjects aged 18-30 years who recorded their food consumption during a week. For infant's intake data, 50 mothers were questioned about the consumption of different milk and food brands during the first year of their babies. All estimated intakes were adjusted for the individual's self-reported body weight (b.wt. = 70 kg; WHO, 1983) and expressed as daily. While infant body weight, during the first 6 months after birth was set at 8 kg as an average (Fohgelberg *et al.*, 2005). AA daily intake calculated according to the following model:

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

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 ($\mu g \text{ kg}^{-1}$). Each data value equally likely on each iteration. Results are summed over foods and individuals.

<div align="center">

RESULTS

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Calibration Curve and Reproducibility

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%.

Analysis of Acrylamide at Low Levels

The extended extraction procedure solve background problems in some matrices and also offers a concentration step by re-extraction of acrylamide in ethyl acetate and evaporation to 1 mL. Additionally, the second extraction step has the advantage that the amount of water used in the first extraction step does not affect the end volume of the prepared extract, e.g., the limit of quantification (LOQ). Figure 2 and 3a-b show typical MRM chromatograms of a baby food samples at different AA concentrations validated down to 7 $\mu g \text{ kg}^{-1}$.
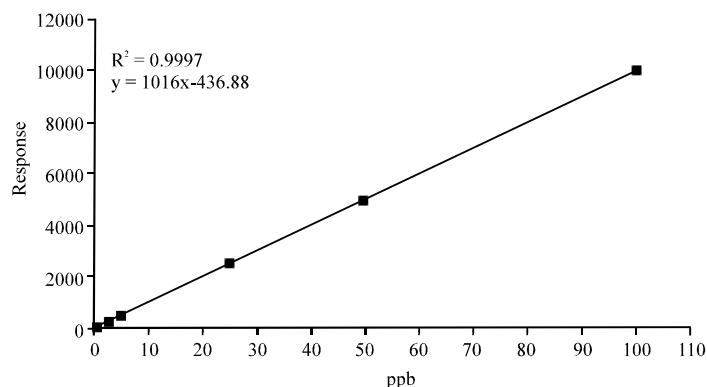
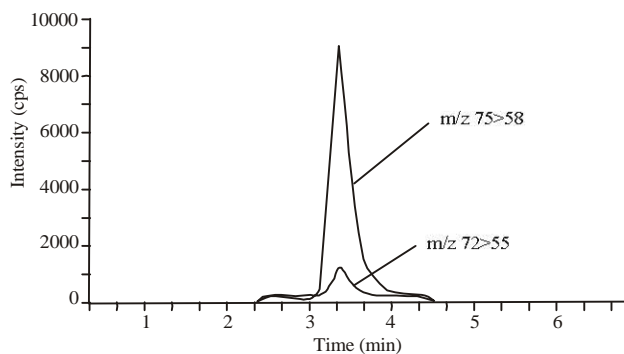

Fig. 1: Caliberation curve of acrylamide

Fig. 2: HPLC-MS-MS analysis of acrylamide in baby food sample (AA content of 10 μg kg^{-1}) MRM chromatogram of a baby food obtained after alternative extraction using HPLC-MS-MS analysis (monitored transitions at m/z 72>55 for acrylamide and 75>58 for D$_3$-acrylamide)

Table 1: Acrylamide (AA) levels in selected food and drink groups in Saudi market

Food group	Sub-group/state	AA level (μg kg^{-1})	
		Present study	Published data
Potato	French fries	206	288[1]
Maize	Extruded (cheese)	319	65-159[2]
	Extruded (salt)	272	
Egg plant	Grilled (Papa Ghanug)	950	NI*
Bread	Shabora	50	NI
	Korsan	90	NI
	Arabian white bread	90	>180[3],10-85[4]
	Arabian dark bread	40	10-85[4]
Biscuit	Sugar Biscuit	220	NI
Cookies	Honey Fetet	50	NI
	Korse Omar	350	NI
	Barazek	40	NI
	Kolijah	40	NI
	Kolijah Haal	60	NI
	Mamul	105	NI
	Teweel	240	NI
Fish	Grilled: out layer	35	NI
	Fried: out layer	17	NI
Sweets	Konaffa	80	NI
	Zalabia	170	NI
	Balah Alsham	40	NI
	Backlawa	72	NI
Date Palm	Hunaini	90	NI
Coffee	Soluble Coffee (Robusta)	820	37-374[1], 3-13[5]; 270[6]; 204[7]
	Roasted Turkish Coffee (Arabica)	290	170-351[8]; 259[9]
	Barely coffee	210	NI

*No Information; [1]Exploratory data on acrylamide in food. USFDA; [2]Pittet *et al.* (2004), [3]Al-Dmoor *et al.* (2004), [4]Olmez *et al.* (2008), [5]Leung *et al.* (2003), [6]Hoenicke *et al.* (2004), [7]Murkovic (2004), [8]Friedman (2003), [9]Svensson *et ai.* (2003)

Level of Acrylamide in Different Foodstuffs

In this study, food samples from twelve food groups subdivided into 28 sub-groups were analyzed for their AA contents (Table 1, 2). The levels of AA varied considerably between single foodstuffs within food groups (Table 1). Regarding the AA levels in
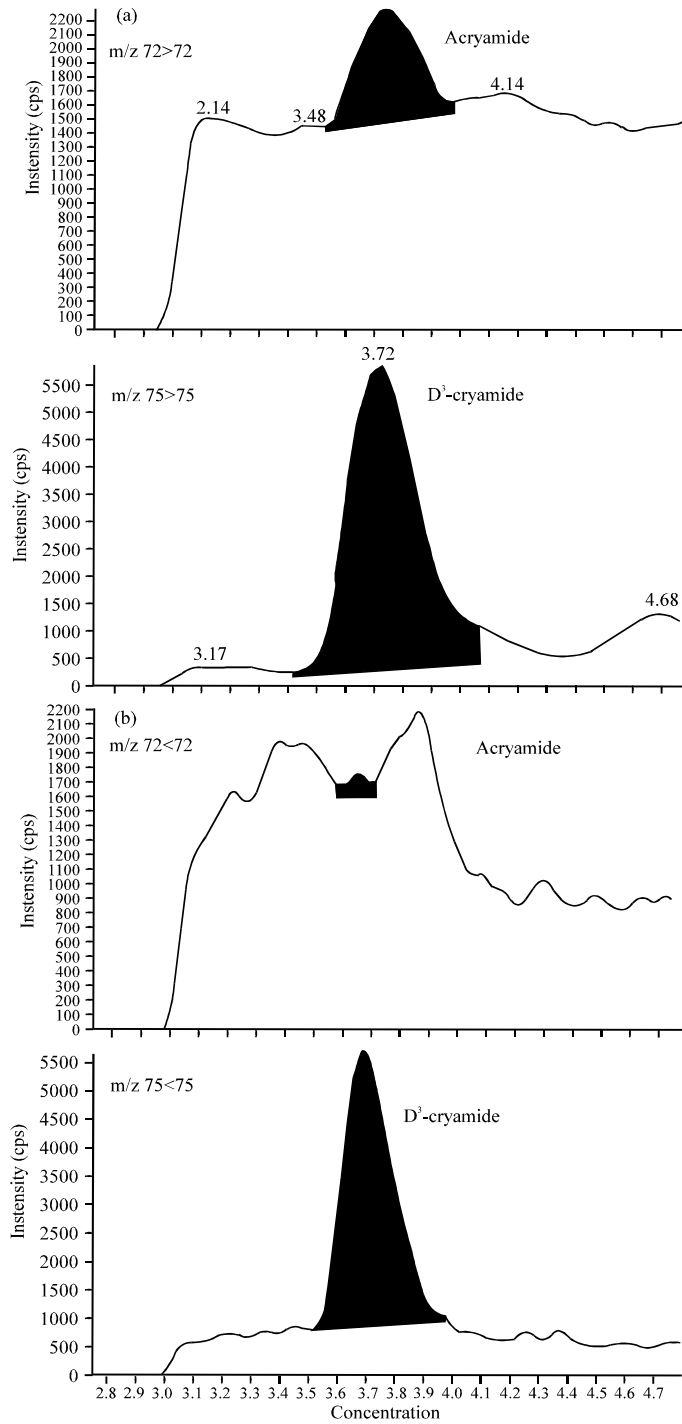
Fig. 3: MRM chromatograms of AA in; (a) powdered baby food (60 μg kg^{-1}) and (b) infant powdered milk (7 μg kg^{-1}) obtained after alternative extraction and HPLC-MS-MS analysis

Table 2: Acrylamide (AA) levels in infant powdered milk and baby foods Saudi market

Food group	Sub-group/State	Brands	AA level ($\mu g\ kg^{-1}$)	AA level ($\mu g\ kg^{-1}$) in Published data
Infant powdered milk	Intended to use after birth	1	4	>10[1], 3.01-9.06[2]
		2	6.5	
		3	3.4	
		4	3.6	
		5	60	
		6	7	
Powdered baby foods	Cereal food	1	13	6.8-124.9[2], 13[3]
		2	10	
		3	24	
		4	30	

[1]Exploratory data on acrylamide in food, USFDA, [2] Jio *et al.* (2005), [3] Canadian data on acrylamide

range at 1000-100 $\mu g\ kg^{-1}$, food groups were ranked as follows, grilled egg plant (950 $\mu g\ kg^{-1}$)>korse Omar; cookies group (350 $\mu g\ kg^{-1}$)>xtruded maize-cheese (319 $\mu g\ kg^{-1}$)>extruded maize-salt (272 $\mu g\ kg^{-1}$)>tweel; cookies group (240 $\mu g\ kg^{-1}$)>sugar biscuit (220 $\mu g\ kg^{-1}$)>French fries (206 $\mu g\ kg^{-1}$) > zalabia; sweets group (170 $\mu g\ kg^{-1}$) and mamul; cookies group. Whereas the rest of analyzed food samples were contained lower AA level amounted to>100- 20 $\mu g\ kg^{-1}$ (Table 1). It is noticed that the white bread contained higher AA than dark bread. The mean AA level in most of traditional cookies types was>100 $\mu g\ kg^{-1}$ however, AA formation is varied depending on flour type, ingredients, temperature/time and layer thickness. Double AA concentration was observed in grilled fish (out layer) in compared to fried fish. Cooked date palm; known as Hunaini, contained a noticeable amount of AA (90 $\mu g\ kg^{-1}$). In regard to coffee drink, highest amount of AA was observed in soluble coffee (820 $\mu g\ kg^{-1}$) followed by roasted Turkish coffee (290 $\mu g\ kg^{-1}$) with lower amount in barely coffee (210 $\mu g\ kg^{-1}$).

Infant powder milk and cereal-milk based food groups were chosen for analysis in this study if they represented a significant part of infant diet. Product sampling was not representative of all brands. Many of the food types are represented by only one product in the survey. Figure 3 showed representative chromatograms of the extracts of both infant powder milk and baby cereal foods. The acrylamide level in infant powdered milk and baby foods were 3.4-60 and 10-30 $\mu g\ kg^{-1}$, respectively (Table 2). Surprisingly, one of infant milk contained an extremely high amount of AA (60 $\mu g\ kg^{-1}$).

Acrylamide Dietary Intake and Daily Exposure

The Arabian white bread followed by coffee group, especially the soluble type compared to Turkish roasted and barely coffee, had the highest contribution to Saudi AA dietary intake (Fig. 4). It is suggested that the sweet group at low level of consumption does not impose health risk with mean 0.4 $\mu g\ day^{-1}$ It is assumed in the worst case intake that a person will consume one portion of each of the twelve food groups involved in this study thus, the average AA dietary intake will be amounted to 60 $\mu g\ AA\ day^{-1}$. This is corresponding to 0.87 $\mu g\ AA\ kg^{-1}$ b.wt. day^{-1} (body weight of 70 kg) which might represent a health risk factor. Figure 5 shows the dietary AA intake from selected brands of infant milk and cereal-milk based foods. The average daily AA intake of different infant milk brands, analyzed in the present study, during the first six months of birth amounted to 0.63 $\mu g\ day^{-1}$. This is corresponding to 0.075 $\mu g\ AA\ kg^{-1}$ b.wt. day^{-1} (body weight of 8 kg). However, a higher intake value (0.9 $\mu g\ day^{-1}$) is expected from the sample had AA level reach to 60 $\mu g\ kg^{-1}$, which is questionable. The mean of the AA daily intake of cereal-milk based infant food found to be higher than infant milk. The average AA intake is amounted to 3.45 $\mu g\ day^{-1}$, which is equivalent to 0.43 $\mu g\ AA\ kg^{-1}$ b.wt. day^{-1}. The accepted level of AA
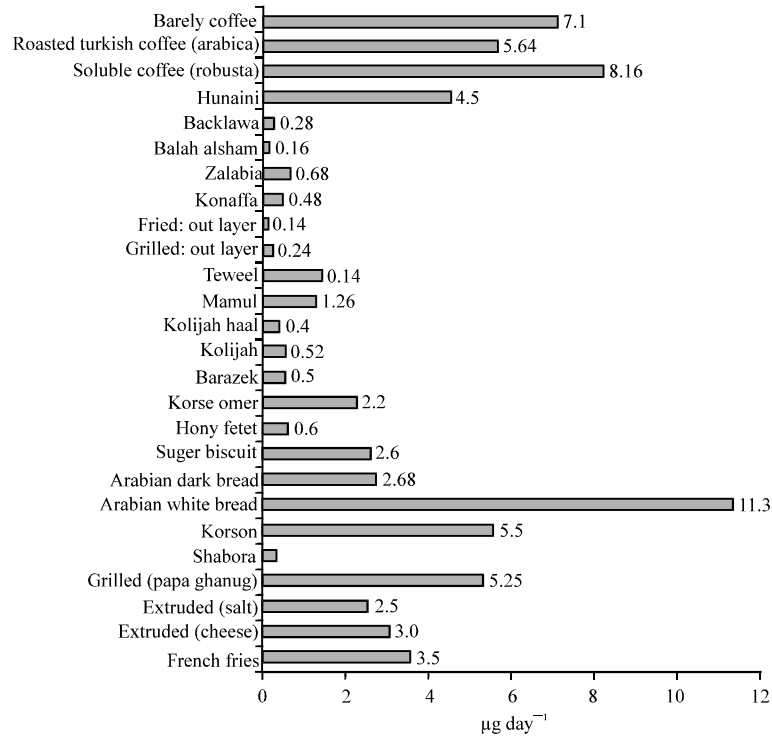
Fig. 4: Dietary intake of acrylamide from selected food product analyzed in the present study
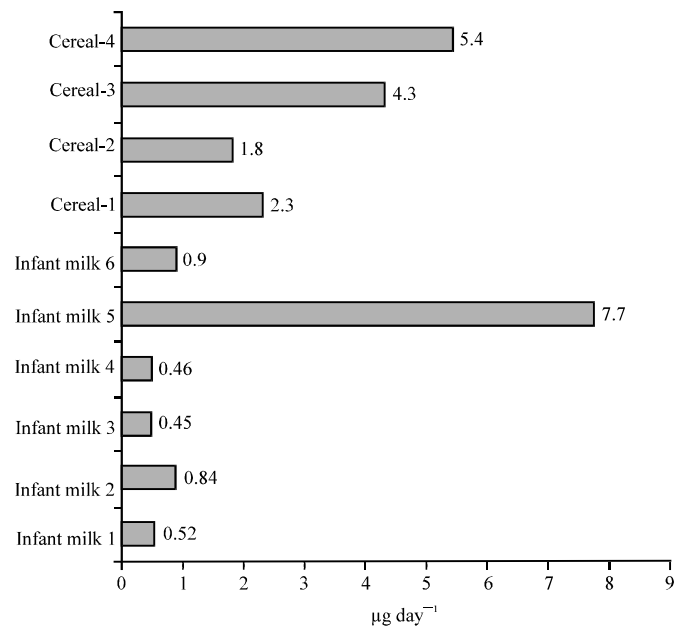

Fig. 5: Dietary intake of acrylamide from selected infant powder milk and cereal-milk based food

dietary intake for infant is not investigated yet. However, by taking the rang of AA dietary intake in adult it is assumed that the rang in infant might be 0.034-0.09 µg kg^{-1} b.wt. day^{-1}.

Hence, the estimated AA intake of infant milk and cereal food might impose health risk and more attention should be employed to reduce the AA level in these products.

DISCUSSION

Analysis of Acrylamide

It has been reported that LC-MS-MS and GC-MS able to determine AA as low as 10 µg kg^{-1} (Taeymans *et al.*, 2004) and 5 µg kg^{-1} (Pittet *et al.*, 2004; Taeymans *et al.*, 2004) respectively. For breast milk, a limit of quantification of 5 ng mL^{-1} using LC-MS-MS was reported (Sörgel *et al.*, 2002). Those various procedures included a wide rang of sample clean-up steps, e.g., protein precipitation and/or extraction by organic solvent or by solid phase cartridges (SPE). Since, AA is very hydrophilic, liquid-liquid extraction from water requires large volumes, in relative terms, of organic solvent. Also, the retention from water on common HPLC and SPE phases is poor. Thus, SPE procedures employed were largely utilized as a chemical filtration, i.e., to remove interfering compounds but not to give a concentrating effect on AA. The sample quantity used for routine extraction is limited to 2 g. A higher amount requires a higher volume of water for extraction because of the swelling properties of most samples. Therefore, the LOQ of the routine method could be not decreased by increasing the weight of the sample. However, using the alternative extraction method 50 mL of water was used. This allows to increase the sample weight and to further increase the concentration level. Consequently, a lower LOQ is achievable. Using 10 g of sample concentrations up to 10 µg kg^{-1} were easily detected in the MRM mode using the transition at m/z 72>55.

The lowest validation levels, where acceptable precision and bias results were obtained, were used as limit of quantification, i.e., 3 µg kg^{-1} for solid samples. In order to confirm the identity of acrylamide in the samples two daughter ions, i.e. the two transitions m/z 72>55 and 72>54, were monitored and the ratio 54/55 was calculated and evaluated as previously described.

The Acrylamide Level

The levels of AA in the data presented here for Saudi market are comparable with previous published data in other countries (Table 1). To our surprise, highest level of acrylamide has been found in Saudi dressing salad (grilled egg-plant), in addition to soluble coffee and some types of biscuits. The present results are in agreement with previous survey studies on AA level in various types of food and drink (Murkovic, 2004; Konings *et al.*, 2003; Svensson *et al.*, 2003). Meanwhile, this is first announcement about AA concentration in traditional Saudi cookies, biscuits, hunaini (cooked palm date with dark flour), sweets and barely coffee. In addition, there are a collection of data originally published by CFSN/FDA, WHO/FAO, and JIFSAN/NCFST. This high variability among the survey data is mainly resulted from the variable heating production processes (°C time^{-1}), variable composition of raw materials and variation in ingredients. It is important to note that present results can not provide guidance in a consumer's choice 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 (Tawfik and El-Ziney, 2008).

The general composition of infant milk formula is included milk proteins (casein and whey proteins) and glucose syrup (reducing sugar) in addition to maltodextrose, lactose and other sugar types which reflect a high possibility of formation of AA especially during the drying process i.e. high temperature and low moisture (Friedman, 2003). Saccharose, a

non-reducing sugar, is only AA forming in higher concentrations. The addition of powder milk also promotes the formation of AA as a result of its lactose content. The use of oligofructoses such as inulin in combination with fructose, sorbitol or on its own is common also in infant formula. Oligofructose also contains small quantities of free fructose and saccharose (Tietz and Habel, 2004). In cereal-based products, acrylamide formation might have occurred as a result of extrusion, baking and roasting process (Studer *et al.*, 2004). 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. Rye has higher asparagine content in comparison with rice, maize and wheat. The extruded products from rye are found to contain higher AA content. By the addition of monosaccharides, disaccharides and oligosaccharides, along with skim-milk powder and malt flour, the AA content can be increased significantly (Kretschmer, 2004). The FDA in USA (USFDA) has analyzed AA levels in several hundred different food products, including baby foods (www.fda.gov). Since, there are different brands with different products on the USA market, it is difficult to compare single AA values given for certain products with the values given in our study. As an example, the mean AA content in four different baby foods (analyzed by USFDA), containing vegetables and different types of meat, was about 20 μg kg^{-1}. The limit of quantification in their survey was higher than reported by Roach *et al.* (2003), 10 μg kg^{-1}, where our results showed big variation in AA level from 4-60 μg kg^{-1}.

The Acrylamide Intake

The FAO/WHO assumes that the range of dietary intake of AA has a range of 0.3-0.8 μg kg^{-1} b.wt. day^{-1} in the developing countries (WHO, 2005). Using the consumption data and analytical AA data in traditional Saudi foods, this is corresponding to 0.87 μg AA kg^{-1} b.wt. day^{-1} (body weight of 70 kg) in Saudi adult which might represent a health risk factor.

Norwegian authorities have reported mean intake values of 0.49 μg kg^{-1} b.wt. day^{-1} for men including coffee consumption. Arabian white bread, soluble coffee, barley coffee and to less extend korsan are the highest drinks and foods contribute to AA dietary intake. It is reported that 39% of AA dietary intake is related to coffee consumption.

The mean milk intake by exclusively breast/substitute-fed infants, from birth up to six months, is estimated to be about 900 mL day^{-1} (Fohgelberg *et al.*, 2005). This is in accordance with other studies. It is estimated that the dietary AA intake for infants during breast-feeding for the first six months is about 0.04 μg kg^{-1} b.wt. day^{-1} (calculating on the basis of a mean body weight of 5.5 kg) while it was 0.5 μg kg^{-1} b.wt. day^{-1} between seven and 12 months. If breast milk substitute is used, as a supplement or instead of breast milk, there will be no measurable differences in the AA intake for the child (Fohgelberg *et al.*, 2005). However, our results diagnosed a higher AA intake level from infant powder milk reached to 0.07 μg kg^{-1} b.wt. day^{-1} and highest intake level from cereal-based food (0.43 μg AA kg^{-1} b.wt. day^{-1}). The estimation of AA intake presented here is reliable since the intake of commercially manufactured cereal-based products is high from six months of age.

Therefore, the presented results here document that the efforts to reduce the exposure to AA in food, thereby minimizing the health risk, should also take into account food products intended for infant. This result should be attached importance to for both food safety departments and correlative infant food manufacturers. For instance, based on such contaminant risk assessment, it can be primarily realized whether modifications in processing and cooking procedures are necessary.

CONCLUSIONS

This study used a LC-MS/MS methodology for the trace quantitative analysis of acrylamide in infant powder milk and baby foods in jars. Especially, this new method is successfully applied to the trace quantification of acrylamide in infant/baby foods, the content of which is less than 10 μg kg^{-1}. A survey of the traditional Saudi foods, and the processed foods included powder infant milk and foods from the Saudi market were performed for determining the AA contents in these products. The traditional Saudi foods and the home-made foods were specifically included in the survey study for AA, so as to use the data in order to evaluate the dietary exposure estimates of the Saudi population. The highest level of AA was determined in Papa Ghanug; dressing salad and korse Omer; type of cookies. In general, soluble coffee, cookies, biscuits, sweets, and fried potato, bread and some of the traditional Saudi desserts were among the food products with high levels of AA. Significant differences were observed in the AA contents of different brands of the infant milk and cereal. Moreover, it is highly required to establish On Line Monitoring (OLM) of AA formation during production process which step is identified as a pre-HACCP requirement.

The present study indicate that the levels of acrylamide in different food groups should be monitored and it is strongly recommended that large-scale research studies regarding the levels of acrylamide in traditional Arabian 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 (WHO, 2002).

ACKNOWLEDGMENTS

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