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## ACRYLAMIDE VARIABILITY AND CONTENT IN SELECTED LOCAL AND IMPORTED FOODS IN JORDAN

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## ABSTRACT

Acrylamide (AC) is a processing contaminant that is formed in some heated starchy food because of Maillard reaction, which involves a reaction between the reducing sugars glucose and fructose, and the amino acid asparagine and there are concerns about its carcinogenicity. In Jordan, limited data is available on AC content in food, especially local and cultural items. Therefore, the present study aimed to estimate the AC content in selected locally produced and consumed food items in Jordan. Acrylamide concentration in 91 selected food items was measured using ELISA kit. In general, the levels of AC in 67% of all food items were over 100 µg/ kg, where AC concentration ranged from 24 – 3403 µg/ kg. Within the bread and bakery products, the highest AC concentration was found in unsweetened Arabic rusk products (kirshalah) ( $268 \pm 36.6$  µg/ kg). In the snack and fast- food groups, the highest mean level was in potato chips ( $1339 \pm 1175$  µg/ kg) followed by fried potato ( $498 \pm 90.5$  µg/kg). Falafel was found to contain ( $106 \pm 21.1$  µg/kg). The concentration of AC in traditional Arabic baked sweets such as kunafeh, baqlawah and haresah had levels of  $198 \pm 3.5$ ,  $101 \pm 14.8$  and  $96 \pm 8.5$  µg/ kg, respectively. The current study provided a base-line data on AC concentration in some Jordanian foods and selected imported snacks. A considerable variation in AC content within each type of locally produced food items was found. The highest AC concentration among the selected local and imported food items in Jordan was in potato products (chips and French fries), followed by biscuits and kirshalleh. Acrylamide monitoring in targeted processed foods should be implemented. Furthermore, an effort needs to be made at national and industrial levels to improve the accuracy and precision of estimated dietary AC intake among different age groups in Jordan.

**Key words:** Acrylamide, processing contaminant, Maillard reaction, ELISA, cereal products, fast food

## INTRODUCTION

Acrylamide (AC), an industrial chemical compound, is a recognized neurotoxic agent, which is considered mutagenic and carcinogenic in animals and as a probable carcinogen in humans [1, 2]. Unexpectedly, AC was detected in a variety of different carbohydrate-rich foods treated with high temperature [3], which alarmed the regulatory authorities and general public about the dietary exposure levels of AC and its metabolites [4, 5]. Previous worldwide food analysis and research data indicate that about 40% of all food commodities contain AC [6-8]. According to the seventy-second report of the Expert Committee on Food Additives (JECFA): potato crisps (chips), coffee extract/ substitute, fried potato, biscuits, bread, cocoa mass/ powder, breakfast cereals and processed cereals/pasta, which comprise the majority of dietary sources, had AC levels of 956, 705, 410, 288, 207, 194, 149 and 127 µg/kg, respectively [9]. Due to the specificity of local and regional food types and processing methods in Jordan, other food items may significantly contribute to AC exposure in the country.

The mechanism of AC formation in food has been the subject of many studies. Efforts have been made to eliminate AC formation through reducing the precursor in food, or by controlling the conditions or factors that influence its production. Accordingly, the amino acid “asparagine” is considered the fundamental precursor in AC formation due to its reaction with a reducing sugar at high temperature via Maillard reaction [10,11]. Several analytical methods are presently available to detect and quantify AC in foods; among them liquid chromatography (LC) and high-performance liquid chromatography (HPLC) with mass spectrometry (MS) are considered the most popular and widely used [12]. However, they are expensive, time consuming and need skilled operators and appropriate sample preparation [13]. Therefore, new rapid screening methods have been developed recently such as Enzyme Linked Immunosorbent Assay (ELISA) to overcome the standard method limitations and provide flexibility and simplicity to food industries [14]. Franek *et al.* [15] evaluated ELISA method for AC detection and reported its sensitivity and reliability, which was consistent with Quan *et al.* [16] evaluation, who considered this method as a reliable, sensitive, low cost and easy to handle alternative.

Information on AC content of local foods in Jordan are limited or not available. Hence, findings of the present study will provide a baseline data for other researchers regarding AC levels in different foods consumed by Jordanians. The objectives of this study were, (1) to analyze AC content of selected Arabic foods and some imported snacks that are available in the market, (2) to explore the

variability in AC content within a food category, and (3) to explore the variability in AC content among food groups.

## MATERIALS AND METHODS

A serological approach using the ELISA assay was used to analyze the AC content of selected Arabic foods and some imported snacks available in the market. A total of 91 food samples were collected from different supermarkets, bakeries and restaurants in Amman, Jordan. To further explore the variability in AC content within a food category and among food groups, these samples were clustered into five groups: Bread and Bakery products, Cereals, Fast Foods, Snacks, and Arabic Bakery Sweets. All food analyses were performed in a duplicate manner.

Acrylamide EIA Kit (Morinaga, Japan) was used for quantitative determination of AC in foods, where the basic assay reagents and solutions were provided in the AC ELISA kit, as well as microplate wells coated with 3-[(2-carbamoyl)ethyl] thio] benzoic acid (3-CTBA). Vertical shaker (OMNI international, GLH-02 homogenizer, USA) and Solid-phase extraction cartridge (SPE) (ISOLUTE Multimode 500 mg/3ml; Biotage Japan LTD) were used in sample preparation and clean up.

Samples were also centrifuged for 20 minutes at 12000 x g (Eppendorf centrifuge; 5804R Germany) for AC extraction. A 96-well microplate reader (Biochrom, ASYS Expert Plus microplate reader, UK) was used to measure the absorbance at 450 nm and 620 nm where the obtained data was processed by computer 4-parameter curve fit.

### A description of locally produced food items is given below

***Kirshalleh and sweet Ka'ak:*** An Arabic Rusk, formed of white flour, milk, oil and leavening agent and baked at 280-300°C.

***Kibbeh:*** A popular Middle Eastern dish that is formed of ground lean beef or lamb meat mixed with minced onions, bulgur and spices, stuffed with cooked minced meat and roasted pine nuts, shaped into balls and finally baked or fried.

***Shawerma:*** it is a traditional fast food in the Arab world and Turkey, which is formed of chicken or beef meat that is roasted slowly on a spit, wrapped in pita bread and served with pickles and garlic sauce.

***Falafel:*** A traditional Middle Eastern fast food, which is a deep-fried patty made from a mixture of ground chickpeas and fava beans and wrapped in pita bread or eaten alone as snack.





**Kunafeh:** A traditional desert which is made of shredded phyllo dough, butter and desalted white cheese, baked in a metal plate and sweetened with sugar syrup.

**Baqlawah:** A rich, sweet pastry made of layers of phyllo pastry and butter, filled with chopped nuts and sweetened with sugar syrup.

**Haresah:** A traditional desert which is made of semolina, milk, sugar and oil, leavened by sodium bicarbonate, baked on metal plate and sweetened with sugar syrup.

**Awwameh:** small dough balls leavened by yeast and fried in vegetable oil then sweetened with sugar syrup.

All samples were homogenized using mortar and pestle, and then exactly 1.00g of the homogenized food sample was placed in a 50 ml centrifugation tube where 19.0 ml of distilled water was added. The sample-water mixture was agitated vigorously at 15000 rpm for 2 min using vertical shaker. Samples were centrifuged for 20 min at 12000×g at 25°C, and the supernatant was harvested in another labeled tube to be cleaned up. The SPE columns were conditioned by washing with 1 ml methanol (once) and distilled water (twice). At this point, 1.0 ml of the samples' supernatant was applied through the conditioned SPE columns, and the effluent discarded since the AC was captured, then, AC was eluted from the columns by rinsing with 3 ml distilled water. Finally, the 3 ml elute were collected in a polypropylene tube and defined with the sample name and date. Acrylamide standard (500 ng/ ml) was diluted with distilled water in sequence by a factor of three using separate 1.5 ml polypropylene tubes, each containing 200 µl. Accordingly, eight different concentrations were obtained and used for ELISA calibration.

Acrylamide shows no immunogenicity due to its low molecular weight (71.08 Dalton) [14], thus, AC should conjugate first with immune stimulating carrier proteins to be able to synthesize the complete antigen. Therefore, the kit used 3-mercaptopbenzoic acid 3-MBA to derivatize AC quantitatively to 3-CTBA. A total of 200 µl of each extracted sample was dispensed into separate 1.5 ml polypropylene tubes, followed by the addition of 40 µl of 3-MBA to all tubes, including the AC standards series. To complete derivatization, all tubes were capped, mixed well, and incubated at 37°C for two hours, followed by adding 240 µl of dilution buffer.

Fifty  $\mu\text{l}$  of derivatized samples and standards were dispensed into 3-CTBA coated ELISA microplate wells, then, 50  $\mu\text{l}$  of "Rabbit Anti- 3- CTBA Antibody" were added to each well. The plate was covered and incubated at 25°C for one hour. Where the antibody binds to the 3-CTBA immobilized on the solid-phase surface, a complex of 3-CTBA/Rabbit anti-3-CTBA antibody is formed. At the end of incubation time, the solution inside the well was discarded and each well was washed six times with 300  $\mu\text{l}$  of washing buffer. To start the second antibody reaction, 100  $\mu\text{l}$  of "Enzyme-conjugated Goat Anti-rabbit IgG Antibody" was dispensed into each well, and again the plate was covered and incubated at 25°C for 30 min to allow the Enzyme-conjugated Goat Anti-rabbit IgG Antibody to react with Rabbit Anti-3-CTBA Antibody on the solid phase. Finally, 3-CTBA/Rabbit anti-3-CTBA Antibody/Enzyme-conjugated Goat Anti-rabbit IgG Antibody complex was formed. The solution in the well was discarded and the washing step repeated. One hundred  $\mu\text{l}$  of Enzyme Substrate (TMB solution) was added to each well and incubated at 25°C for 30 min where a blue color developed due to the action of the enzyme bound to the surface of the well. To stop the reaction, 100  $\mu\text{l}$  of (1 N Sulfuric Acid) were added to each well, and the color turned to yellow. The absorbance was measured at OD 450 nm and OD 620 nm with a 96-well microplate reader. The average absorbance of duplicate determinations ( $A_{450}$  minus  $A_{620}$ ) was calculated and the AC concentration in  $\mu\text{g/l}$  were obtained from the calibration curve, then multiplied by dilution factor to get the final result in  $\mu\text{g/kg}$ . Samples of cornstarch were spiked with AC at 500, 250, 200 and 150 ng/ kg, and then extracted and analyzed according to the previous ELISA procedure to detect the recovered AC concentration.

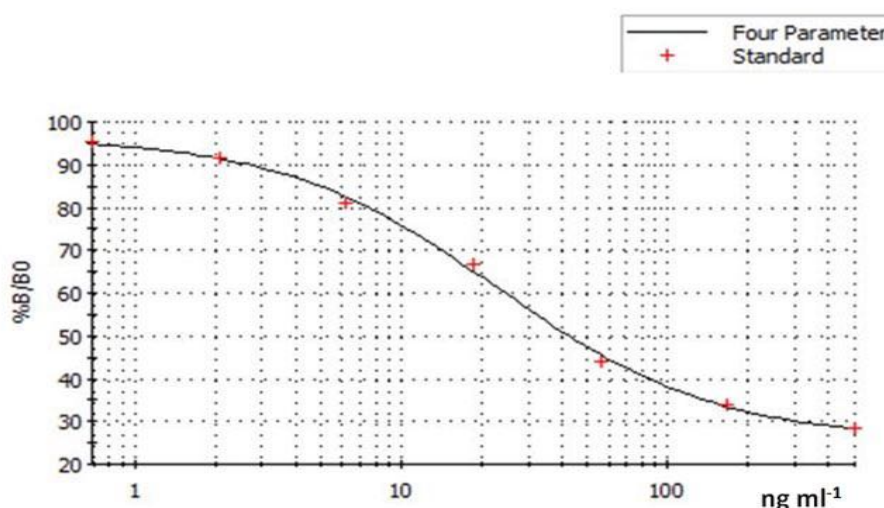
## RESULTS AND DISCUSSION

The established assay sensitivity in this study was determined as the lowest concentration that can be distinguished from zero [Limit of detection (LOD)], which was 0.4 ng/ ml (=24  $\mu\text{g/kg}$ ). The standard calibration curve for the ELISA was constructed from 7 calibration standards and transformed into a semi-logarithmic graph model (Figure 1). The value of the correlation coefficient ( $R^2$ ) that was obtained from this model was 0.9961, which represented a good agreement with the experimental data and indicated a good fitting power for the model. The precision of the assay was determined using the spiked samples and illustrated in Table 1 as the percentage of recovery, ranging from 84% to 104.5%.

In the selected 91 food items, AC levels ranged from 24 to 3403  $\mu\text{g/kg}$  (Table 2), where only two samples (Zinger and Asabe' Zainab) had AC content below LOD. The highest AC mean level was in commercial potato chips ( $1339 \pm 1175 \mu\text{g/kg}$ )



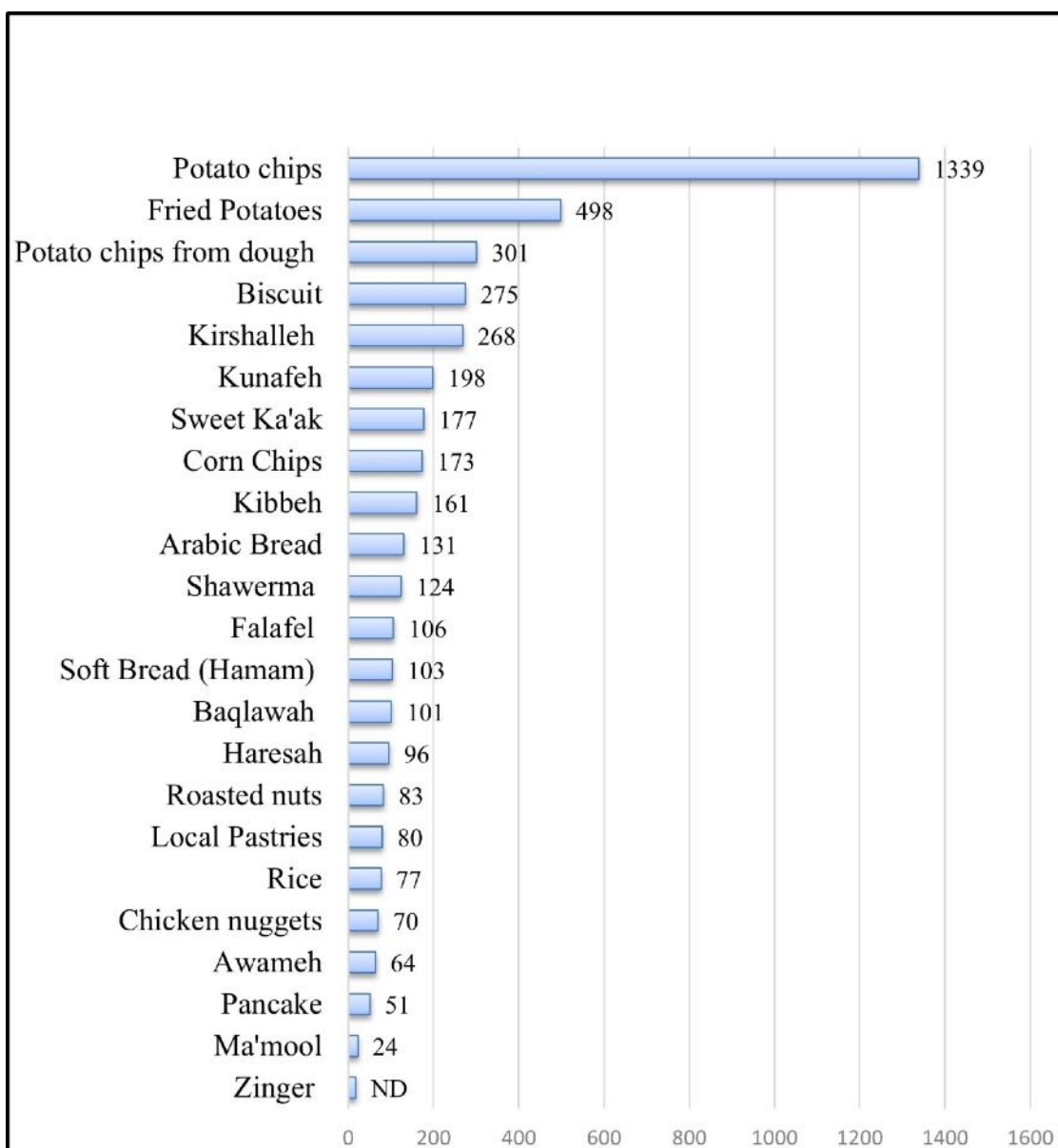
followed by fried potatoes ( $498 \pm 90.5 \mu\text{g/ kg}$ ), while the lowest concentration detected was in Ma'amool (traditional butter cookies filled with dates or nuts) ( $24 \mu\text{g/ kg}$ ) (Figure 2). Falafel, the highly consumed fast food in Jordan, was found to contain  $106 \pm 21.1 \mu\text{g/ kg}$  (Figure 3), whereas the traditional Arabic baked sweets like kunafeh, baqlawah, haresah and awwameh had AC mean levels of  $198 \pm 3.5$ ,  $101 \pm 14.8$ ,  $96 \pm 8.5$  and  $64 \pm 11.3 \mu\text{g/ kg}$ , respectively (Table 2). The current study provided a base-line data on AC concentration in some Jordanian foods and selected imported snacks, where the analyses of these food items may help to improve the accuracy and precision of estimated dietary AC intake among different age groups.



**Figure 1: The standard calibration curve transformed into semi-logarithmic graph**

Semi-logarithmic graph of the calibration curve was drawn, where the horizontal (logarithmic) and vertical (linear) are concentration (ng/ml) of acrylamide in standard and reading values of absorbance (B/B0), respectively.





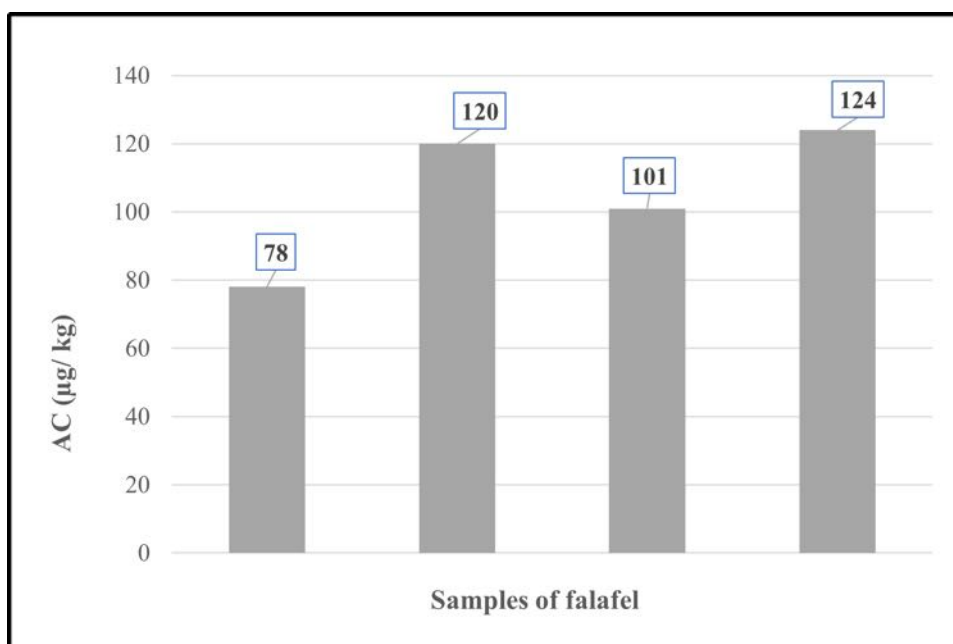
**Figure 2: Acrylamide levels (µg/ kg) in selected food items**

Each column represents acrylamide concentration in µg/ kg, from the highest to the lowest in selected food items. (ND: not detected)

Levels of AC in potato chips (crisps) were the highest ( $1339 \pm 1175$  µg/ kg). This AC content was comparable to previously published international data by FAO/WHO, which included 878 samples of potato chips with an average of 956 µg/ kg [9].

A comparison of the current findings with those of other studies confirms that potato chips show the highest concentrations of AC among other food items. In a study published by Mencin *et al.* [17], high AC levels were detected in Slovenian

crisps (3439  $\mu\text{g/kg}$ ) and potato sticks (3102  $\mu\text{g/kg}$ ), however, these values were higher than the present findings. Furthermore, potato snacks from the Korean market showed a lower mean value of AC (554.5  $\mu\text{g/kg}$ ) according to Jeong *et al.* [18]. According to Saleh and El-Okazi [18], the AC level in Egyptian potato chips was  $1500 \pm 645$   $\mu\text{g/kg}$ , which is consistent with the present data. In contrast, Husamo *et al.* [20] reported a mean AC level in Syrian potato chips of 469.3  $\mu\text{g/kg}$ , whereas the mean of 280 potato chips samples was 575  $\mu\text{g/kg}$  in Europe [6]. Figure (2) illustrates the great variation in AC concentration among selected food items. Moreover, considerable variation in AC content was also found within each type of food item. The AC level in the six bread samples ranged from 97 – 164  $\mu\text{g/kg}$  (Table 2). Similarly, the AC level in six samples of hamam bread was between 97 – 111  $\mu\text{g/kg}$ . The AC range in samples of falafel, potato chips, and corn chips is shown in figures 3 to 5, respectively.



**Figure 3: Variability of acrylamide levels in falafel samples**

Columns show the variability in acrylamide (AC) concentration in six different samples of locally produced falafel from different places

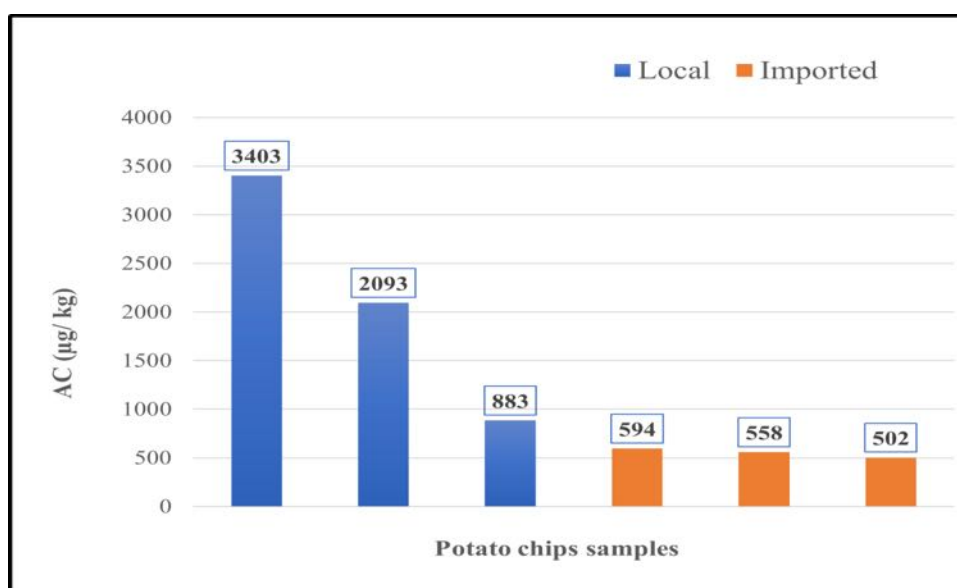
The present data were characterized by large variability, especially for potato crisps that showed the widest range of concentrations (Figure 4). Unfortunately, in the reported results, the AC level in locally manufactured potato chips was four fold higher than that in the imported items (2126 compared with 551  $\mu\text{g/kg}$ ) (Figure 4). Similarly, AC content in locally produced corn chips was two-fold higher than that in the imported brands (218.5 compared to 113  $\mu\text{g/kg}$ ) (Figure 5), which highlights the need to control and improve the production conditions for those products.

The mean AC level in deep-fried potato (French fries) in the current study was  $498 \pm 90.5 \mu\text{g}/\text{kg}$ , which is also consistent with data from FAO/WHO [6] that reported an AC level of  $410 \pm 508 \mu\text{g}/\text{kg}$ . Correspondingly, EFSA [9] reported that AC concentration in French fries ranged from 318-311  $\mu\text{g}/\text{kg}$ . Saleh and El-Okazi [19] previously reported an AC concentration of  $540 \pm 213 \mu\text{g}/\text{kg}$ . Nevertheless, the observed large variation in AC levels in both mentioned potato products can be explained by the variability in raw materials varieties and storage conditions (below  $8^\circ\text{C}$ ). These variables can affect the concentration of AC precursors, which are asparagine and reducing sugar [21]. Other explanations could be related to variations in cooking conditions such as temperature, time of cooking, and the size of potato pieces [7, 22].

In this study, AC contents in Arabic bread, Hamam bread, Sweet Ka'ak and Kirshallah were  $131 \pm 21.6$ ,  $103 \pm 5.6$ ,  $177 \pm 42.1$ , and  $268 \pm 36.6 \mu\text{g}/\text{kg}$ , respectively (Table 2). Although the samples were obtained from six different bakeries in Amman, the variation between these different samples of the same product relatively was not high (small SD) (Table 2). The observed limited variation might be due to the use of similar recipes and baking conditions. In this context, it is believed that the type of oven and time of fermentation affect AC content [24-25]. Conversely, the only previously reported data in Jordan indicated that AC concentration in Arabic bread, Hamam bread, Sweet Ka'ak and fermented Kirshallah was  $< 180$ ,  $3300$ ,  $4700$ , and  $4300 \mu\text{g}/\text{kg}$ , respectively [26], which are not comparable to the present data. These inconsistencies could be due to different extraction and analyses methods. However, AC content in bread as reported by FAO/WHO was  $207 \mu\text{g}/\text{kg}$  [9], and  $54 - 68 \mu\text{g}/\text{kg}$  in soft bread as reported by EFSA [6], which are comparable with the current data. The levels of AC reported in this study are consistent with the data observed in toast and bread from the Slovenian market that ranged from  $44.5 \mu\text{g}/\text{kg}$  to  $246.1 \mu\text{g}/\text{kg}$  [17]. In contrast, they are higher than AC concentrations in Korean bread, which ranged from ND to  $48.8 \mu\text{g}/\text{kg}$  [18].

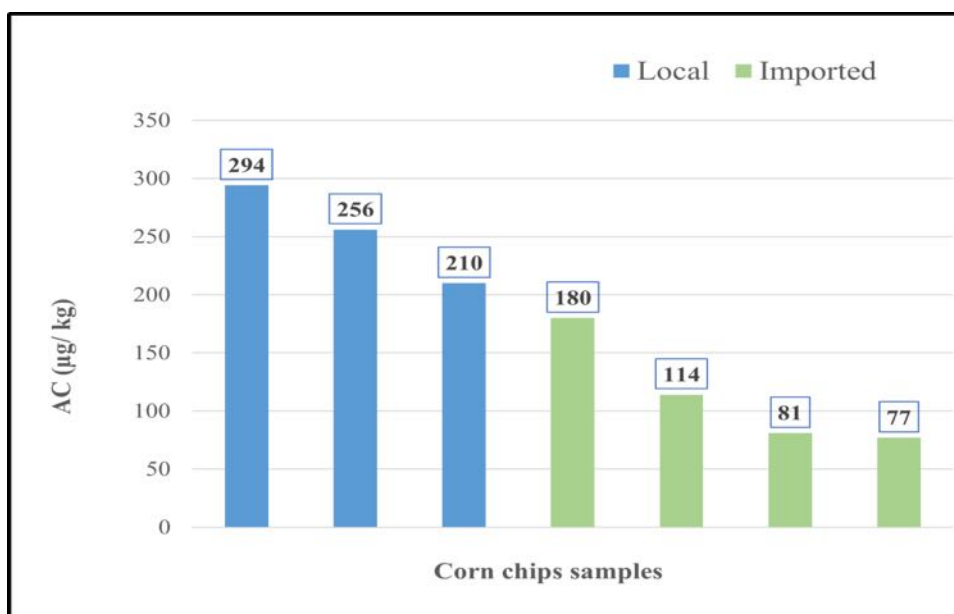
As indicated in figure (3), the average AC content from four different falafel samples was  $106 \pm 21.1 \mu\text{g}/\text{kg}$ , which is lower than AC contamination in other regional countries such as Syrian and Egyptian falafel ( $155$  and  $273 \mu\text{g}/\text{kg}$ , respectively) [19,20]. The differences could be attributed to the variation in recipe ingredients and proportions. Another possible factor could be the size of the fried patty in addition to the frying temperature and type of oil used.

In the present study, six different Arabic sweets were analyzed (Table 2), among them kunafeh was found to contain the highest AC concentration (189  $\mu\text{g}/\text{kg}$ ), followed by baklawa (101  $\mu\text{g}/\text{kg}$ ) and Haresah (96  $\mu\text{g}/\text{kg}$ ). Considerable variations between these values and Al-Dmoor *et al.* [26] results for the same items, (2900, 4600, 4200  $\mu\text{g}/\text{kg}$ ), respectively were observed. This observation could be explained by the fact that a different chemical assay was used in the present study.



**Figure 4: Variability of acrylamide levels in potato chips samples**

It represents the variability in acrylamide (AC) concentration of potato chips, and it compares these variabilities between the imported and the locally produced products as well

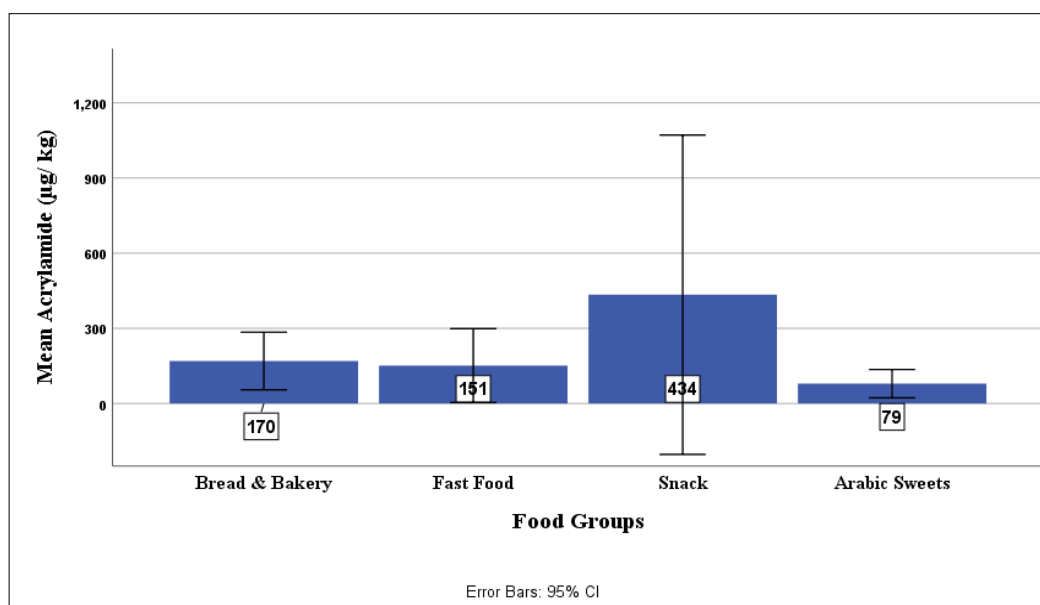


**Figure 5: Variability of acrylamide levels in corn chips samples**

It represents the variability in acrylamide (AC) concentration of corn chips, and it compares these variabilities between the imported and the locally produced products as well

To explore the AC variability among food groups, the mean value for the AC concentration in each food group was listed and presented in table (2). Further, the mean value for the AC concentration in each food group was also calculated and reported in figure (6). As expected and consistent with other studies, the snack group had a higher AC mean value (434 µg/ kg) due to the presence of potato chips, while the mean value for bread and bakery, fast food, and Arabic sweets were 170, 151, and 79µg/ kg, respectively (Figure 6).





**Figure 6: Variability of acrylamide levels among five different food groups**

This figure represents the variability in acrylamide (AC) concentration among food groups, where the snack group contains the higher mean level of AC (434 µg/kg), followed by the Bread and bakery group (170 µg/kg) and the fast food group (151 µg/kg)

Although it would be hard to eliminate AC from food entirely, reducing AC levels in foods might mitigate human health risks from exposure to AC. Therefore, international attempts have concentrated on recognizing techniques that can lower AC in foods and publishing that information in the form of practice codes, guidance and toolboxes [6, 9, 27, 28]. Comments obtained by FDA referred to the various aspects causing variability in AC levels and the challenges in controlling this variability. These aspects indicated seasonal changes in the composition of raw materials, differences in product formulation and processing equipment, and variations in other cooking parameters. Accordingly, FDA assumed that it would be applicable to build guidance with information on lowering AC levels in foods [27, 28]. The FDA Guidance for food industry suggests a range of possible approaches to reducing AC levels without identifying specific recommended approaches [27]. This guidance encouraged growers, manufacturers and food service operators to assess approaches that may be applicable to their individual processes and to consider implementing approaches that decrease AC levels in their products [27, 28].

## CONCLUSION

The present study established a baseline data on AC concentration in some Jordanian foods and selected imported snacks. Potato products (chips and French fries) contain the highest AC concentration among the selected local and imported food items in Jordan, followed by biscuits and kirshalleh. Wide variation in AC content in different brands of the same food item was demonstrated, where higher levels of AC were detected in local brands of potato and corn chips and snacks compared to the imported brand from the same categories. Unfortunately, food composition tables that provide the AC composition of foods to evaluate AC dietary exposure are limited. Although many countries such as the United States, the United Kingdom, China, Germany, Canada, Japan, and others have a food composition reference, the capacity of these databases to show updated AC composition of the food items is still being determined. Acrylamide variability among food groups in the present study was considerable. The snack group had the highest AC mean concentration due to the presence of potato chips, which constitutes the highest AC concentration among all selected food items. Further research is needed to assess the dietary exposure levels of AC among the Jordanian population and to explore its health impact. It is recommended to promote prevention and elimination strategies within the food industry to limit AC within processed food. Monitoring of AC in targeted processed foods should be implemented including cooking method, time and temperature.

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**Table 1: Percentage of the recovery for ELISA assay**

	Spiked samples			
	150 µg/ kg	200 µg/ kg	250 µg/ kg	500 µg/ kg
Results (µg/ kg)	139.6	208.9	251.6	417.7
% Recovery	93.10%	104.50%	101%	84%

**Table 2: Acrylamide mean concentrations in selected food items**

Food Group	Food Items (Subgroup)	AC content µg/kg *
<b>Bread and Bakery products (n=24)</b>	Arabic Bread (n=6)	131 $\pm$ 21.6
	Soft Bread (Hamam) (n=6)	103 $\pm$ 5.6
	Sweet Ka'ak (n=6)	177 $\pm$ 42.1
	Kirshalah (unsweetened) (n=6)	268 $\pm$ 36.6
<b>Cereals (n=4)</b>	Rice (n=4)	77 $\pm$ 12.9
<b>Fast Food (n=25)</b>	Fried Potatoes (n=10)	498 $\pm$ 90.5
	Falafel (n=4)	106 $\pm$ 21.1
	Shawarma (n=2)	124 $\pm$ 0.5
	Zinger (n=2)	< LOD
	Chicken nuggets (n=2)	70 $\pm$ 7.1
	Kibbeh (n=2)	161 $\pm$ 5.7
	Local Pastries (n=3)	80 $\pm$ 18
<b>Snacks (n=24)</b>	Potato chips (n=6)	1339 $\pm$ 1175
	Potato chips from dough (n=3)	301 $\pm$ 114
	Corn Chips (n=7)	173 $\pm$ 86
	Biscuit (n=6)	275 $\pm$ 211
	Roasted nuts(n=2)	83 $\pm$ 11.3
<b>Arabic Sweets (n=14)</b>	Kunafeh (n=2)	198 $\pm$ 3.5
	Baklawas (n=2)	101 $\pm$ 14.9
	Haresah (n=2)	96 $\pm$ 8.5
	Awameh (n=2)	64 $\pm$ 11.3
	Asabe' Zainab (n=2)	< LOD
	Ma'mool (n=2)	24 $\pm$ 0
	Pancake (n=2)	51 $\pm$ 10.6
<p>* Data are presented as mean <math>\pm</math> standard deviation (SD)</p> <p>LOD: Limit of detection</p> <p>n : number of samples</p>		

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