

Original Research Article

Determination of acrylamide levels in selected commercial and traditional foods in Syria

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Abstract

Purpose: To determine acrylamide (AA) levels in different brands of commercial and traditional foodstuffs available in Syria by ultra-performance liquid chromatography-mass spectrometry (UPLC-MS).

Methods: A total of 63 samples were analyzed. Food samples were defatted by hexane and then extracted with methanol 98 % in a vortex mixer. Thereafter, Carrez I and Carrez II were added to precipitate proteins from the co-extractives and then centrifuged to obtain a clear aqueous extract that was evaporated to dryness. The extract was dissolved in 1 mL of water, eluted through a preconditioned Oasis HLB cartridge and then filtered. The filtrate was analyzed by UPLC-MS/MS to determine AA content.

Results: Among the commercial foods tested, the highest acrylamide quantity was found in potato products ($396 \pm 3.59 - 1844 \pm 5.29 \mu\text{g kg}^{-1}$) and the lowest in corn products ($183 \pm 2.64 - 366 \pm 4.58 \mu\text{g kg}^{-1}$). This was followed by biscuits ($57 \pm 2.64 - 1433 \pm 2.51 \mu\text{g kg}^{-1}$), breakfast cereals ($121 \pm 8.73 - 245 \pm 3.60 \mu\text{g kg}^{-1}$), bread ($119 \pm 1.73 - 263 \pm 3.60 \mu\text{g kg}^{-1}$) and then coffee ($113 \pm 2.64 - 64 \pm 3.05 \mu\text{g kg}^{-1}$). Regarding traditional foods, the highest level of AA was found in AL-Mshabak ($481 \pm 2.08 \mu\text{g kg}^{-1}$) and AL-Awamat ($421 \pm 2.64 \mu\text{g kg}^{-1}$) followed by AL-Namora ($282 \pm 4.35 \mu\text{g kg}^{-1}$) and AL-Kenafa ($242 \pm 2.64 \mu\text{g kg}^{-1}$). It was also observed that the lowest amount of AA was in fried bread ($230 \mu\text{g kg}^{-1}$), AL-Fatayer ($192 \pm 3.51 \mu\text{g kg}^{-1}$) and AL-Baqlawwa ($172 \pm 4.35 \mu\text{g kg}^{-1}$) while Eid Aqras ($130 \pm 4.58 \mu\text{g kg}^{-1}$) and AL-Brazeq ($167 \pm 3.78 \mu\text{g kg}^{-1}$) contained the least amount of AA.

Conclusion: The results indicate that the highest levels of AA are found in the most commonly consumed foods. There was significant difference ($p < 0.05$) in AA levels among different food items and within different brands of the same product.

Keywords: Acrylamide, Commercial foods, Traditional foods, Syrian food, Contaminants, UPLC-MS/MS

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INTRODUCTION

The occurrence of elevated levels of acrylamide (AA) in fried and baked carbohydrate-rich foodstuffs was investigated and reported by the Swedish National Food Administration, Stockholm University in 2002 [1]. Based on these

findings a series of further investigations were carried out by a number of international organizations, e.g., World Health Organization, and Food and Agriculture Organization of United Nations [2], which detected AA in various foodstuffs. AA has now become a public health concern as it is one of the most common food

contaminants in processed food at high temperature [3], with neurotoxic carcinogenic and genotoxic effects [5] and classified Group 2A carcinogen [6] and category 2 mutagen. Over the years, efforts have been carried out to build a deeper understanding of AA formation in foodstuff with evidence suggesting that there could be numerous mechanisms by which AA is formed. Among these possibilities is the reaction between free asparagine (Asn) and a carbonyl source resulting in the reduction of sugars (i.e., glucose and fructose) by the Maillard reaction at a high temperature [7]. Alternatively, the reaction between acrylic acid and ammonia can form AA in a lipid rich food [8]. Although different analytical techniques such as high-performance liquid chromatography (HPLC), gas chromatography (GC) or liquid chromatography (LC) were successful in the determination of the AA content in food, the ultra-performance liquid chromatography (UPLC) method has the advantage that the resolution is improved with higher analytical sensitivity and a shorter retention time [9].

Various research and quantifications of AA in foodstuffs have been carried out in many countries e.g., Hong Kong [10], North America and European countries [11], Japan. [12], Australian [13], Caribbean countries [14], Spain [15], Poland [16], China [17] and India [18] but until now, only one paper has been published on AA levels in fried potato and falafel from Syria even though Syrians consume considerable amounts of baked and fried traditional foodstuffs. Therefore the main objective of this study was to determine the AA content in various commercial and traditional Syrian foods by a UPLC method and to increase public awareness of the results.

EXPERIMENTAL

Sampling

A total of 63 samples were analyzed for toxic AA content. The studies that were carried out on the acrylamide content in food in different countries reported that the main contributing foods are: sweet biscuits (10 – 20 %), french fries (6 – 46 %), coffee (13 – 39 %), potato crisps, pastry and potato chips (16 – 30 %), and bread and rolls/toasts (10–30 %) with other food sources contributing less than 10 % of the total exposure [19]. Therefore, according to these data, food sample selection was focused mainly on these products. Likewise, to assess AA levels in traditional foods, a selection of foods that are part of the Syrian diet and that may contain AA

were investigated. All food products were purchased from different local supermarkets in the city of Homs, in the Central Western regions of Syria in 2015. All the samples were immediately sealed in polyethylene bags and stored at 4 °C while the homemade traditional food was prepared according to traditional methods. Each type of food was analyzed in triplicate.

Preparing of traditional food

AL-Kenafah is a crispy sweet made by using threads of vermicelli and prepared from a white flour mixture. The threads are put on to a metal plate and spread out and then the filling is added which is desalted white cheese. It is then covered with more threads of vermicelli and baked on a flame heater at 180 – 200 °C for 10 min. It is sweetened after baking with a heavy sugar syrup, making 40 % of the whole food a sweet crust.

AL-Awamat are small dough balls, which are fried like doughnuts. They are leavened by yeast for 10 min before frying in vegetable oil at 160-180 °C for 6-8 min. After frying they are sweetened by dipping in heavy sugar syrup.

AL-Namora are composed of fine semolina, sugar and milk, leavened by sodium bicarbonate and baked at 200 - 250 °C for 25 min before sweetening with heavy sugar syrup.

AL-Brazek is a cracker type bakery product consisting of a crust made of white flour, sugar, eggs, butter and some orange juice. It is mixed well and leavened with sodium bicarbonate, covered by sesame and then baked on a flame heater at 180 – 200 °C for 15 min.

AL- Mshabak are fried dough sticks made from coarse semolina flour, leavened with yeast and deep fried in vegetable oil at 160-180 °C for 6-8 min after which they are sweetened by dipping in a heavy sugar syrup.

Chemicals and standards

Acrylamide standard (> 99 %) was purchased from Augsburg, Germany, while 13C3-acrylamide (99 % isotopic purity) was obtained from Cambridge Isotope Laboratories Inc. (Andover, USA). Methanol 98 %, hexane, potassium hexacyanoferrate and zinc sulfate were obtained from Sinopharm Chemical Reagent Co., Ltd (SCRC) (China). Solid phase extraction cartridges (Oasis MCX and Oasis HLB, 1 mL) were supplied by Waters Corp (Berkshire, England). All used solvents were of

HPLC quality and other used chemicals were of analytical grade.

Standard solutions

All AA stock and working solutions were prepared in methanol 98 %. A standard AA stock solution (1.0 mg/mL) was prepared by dissolving 1.0 mg of AA in 1.0 mL of methanol 98 %. The stock solutions were diluted with methanol 98 % to give a series of standards 50, 100, 200, 300, 400, 500, 1000, and 2000 mg/ L. All standard solutions were stored in amber narrow-mouth bottles at 48 °C.

Carrez 1 solution was prepared by dissolving 15 g of potassium hexacyanoferrate in 100 mL distilled water. Carrez 2 solution was prepared by dissolving 30 g of zinc sulfate in 100 mL distilled water.

Sample preparation

The food samples were ground homogeneously to less than 1 mm mesh size prior to AA extraction. Ground sample of 1 g was weighed in a 50 mL centrifuge tube and defatted by adding hexane and then extracted with 9 mL of methanol 98 % for 5 min in a vortex mixer whilst 100 μ L Carrez I and 100 μ L Carrez II were added to the centrifuge tubes to precipitate proteins from the co-extractives and then centrifuged at 8000 rpm for 5 min. After centrifugation, the optically clear aqueous extract was transferred to a tube and the solid residue was re-extracted a further two times to increase the extraction yield of AA but without further use of Carrez solution. The combined extracts were evaporated to dryness under a gentle stream of nitrogen (< 3 psig). The residue was reconstituted in 1 mL water and eluted through a preconditioned Oasis HLB cartridge at a rate of one drop per second. The first drops of the elute were discarded while the remaining drops were collected and filtered through a 0.22 mm nylon filter prior to LC–MS analysis.

UPLC MS/MS analysis

The AA content in a pretreated sample was determined by UPLC-MS/MS with electrospray positive ionization (ESI+). Furthermore, an ACQUITY UPLC quaternary pump system equipped with micro vacuum degasser, thermostated autosampler and thermostated column compartment (Waters, Milford, MA, USA) was coupled with a Micromass Quattro Ultima triple-quadrupole mass spectrometer from Micromass Company Inc. (Manchester, UK). Sample extracts (injection volume: 10 μ L) were

injected in a UPLC BEH C18 column (Waters, Milford, MA, USA) and the column temperature was set at 25 °C while the column length was set at 100 mm with a run time of 3 min and a flow rate maintained at 0.5 mL/min. The conditions used for the electrospray source were as follows: source temperature, 120 °C; cone voltage, 35 V; desolvation gas temperature, 350 °C; capillary voltage, 3.5 kV; desolvation gas flow, 400 L/h nitrogen; cone gas flow, 45 L/h nitrogen; argon collision gas pressure to 3×10^{-3} mbar for MS/MS. The mobile phase was 10 % methanol/0.1 % formic acid in water with a flow rate of 0.3 mL/min.

Statistical analysis

The results are presented as mean \pm standard deviation (SD). The statistical analyses were performed on Statistical Package of Social Sciences (SPSS) version 20 using one way analysis of variance (ANOVA) followed by Dunnett's method to compare the groups. $P \leq 0.05$ was considered statistically significant.

RESULTS

Limit of detection (LOD), limit of quantification (LOQ) and recovery

Recovery of the method was determined by adding different concentrations of AA to a boiled potato sample followed by the sample extraction steps and UPLC analyses under the same conditions. A percentage recovery of > 90 % was obtained.

Acrylamide content

A total of 63 commercial food products and traditional foods obtained from Syrian retail stores were analyzed. The median and the range of the AA levels in these products are showed in Table 1 and table 2 showing the first significant variability of AA levels $p \leq 0.05$ among different foodstuffs, including bakery products, potato products, corn products, coffee, and traditional foods. This was also observed within different brands of the same product. Regarding bakery products, the AA levels in bread samples were found to be $153 \pm 2.64 \mu\text{gkg}^{-1}$ in Syrian whole wheat bread and $119 \pm 1.7 \mu\text{gkg}^{-1}$ in Syrian white flour bread. A higher AA content of $263 \pm 3.60 \mu\text{gkg}^{-1}$ was found in soft bread while bread sticks and salty bread sticks contained $229 \pm 4.35 \mu\text{g kg}^{-1}$ and $190 \pm 2.51 \mu\text{gkg}^{-1}$ respectively. Acrylamide levels were detected in the range of $74 \pm 3.64 - 1433 \pm 2.51 \mu\text{gkg}^{-1}$ wheat biscuit-related products (biscuit and wafer) and lower

Table 1: Acrylamide content ($\mu\text{g kg}^{-1}$) of some commercial food products

Food group	Acrylamide range	Median	Number of samples
Coffee	64±3.05–121±4.58	192 ^a	44
Corn biscuits, wafers, crackers	57±2.64–325±1.96	160 ^b	77
Breakfast cereals	121±8.73 – 245±3.60	167 ^c	33
Bread	119±1.73–263±3.60	190 ^d	66
Corn chips	183±2.64–366±4.58	249 ^e	6
Wheat biscuits, wafers, crackers, crackers	74±3.64 – 1433±2.51	431 ^f	10
Potato chips	396±3.59–1844±5.2	1034 ^g	99

Note: The results are presented as mean \pm standard deviation (SD) ($n = 3$); a–g values are significantly different from each other and significance accepted at $p \leq 0.05$

AA concentrations of $57 \pm 2.64 - 325 \pm 1.96 \mu\text{gkg}^{-1}$ were found in corn biscuit-related products (biscuit and wafer) and $121 \pm 8.73 - 245 \pm 3.60 \mu\text{gkg}^{-1}$ in breakfast cereals.

The study showed that the concentrations of AA in different brands of potato products ranged between 396 ± 3.59 and $1844 \pm 5.29 \mu\text{gkg}^{-1}$ while in different brands of corn chip products AA content was between 183 ± 2.64 and $366 \pm 4.58 \mu\text{g kg}^{-1}$. Regarding traditional foods, the highest levels of AA were found in AL- Mshabak ($481 \pm 2.08 \mu\text{gkg}^{-1}$) and AL-Awamat ($421 \pm 2.64 \mu\text{gkg}^{-1}$) while the lowest concentrations were detected in AL-Namora ($282 \pm 4.35 \mu\text{gkg}^{-1}$) and AL-Kenafa ($242 \pm 2.64 \mu\text{gkg}^{-1}$) followed by fried bread ($230 \pm 4.06 \mu\text{gkg}^{-1}$), AL-Fatayer ($192 \pm 3.51 \mu\text{gkg}^{-1}$) and AL-Baqlawwa ($172 \pm 4.35 \mu\text{gkg}^{-1}$). However, it was also found that the lowest levels of AA were in Eid Aqras ($130 \pm 4.58 \mu\text{gkg}^{-1}$), and AL-Brazeq ($167 \pm 3.78 \mu\text{gkg}^{-1}$).

Different coffee brands were also analyzed to detect AA content with the results of $113 \pm 2.64 - 121 \pm 8.73 \mu\text{gkg}^{-1}$ in Medium roasted ground coffee and $64 \pm 3.05 - 71 \pm 2.33 \mu\text{gkg}^{-1}$ in dark roasted coffee.

Acrylamide levels in traditional Syrian foods

AA levels in most commonly consumed commercial and home-made traditional Syrian foods are showed in Table 2, including fried and baked products.

The lowest AA contents were detected in Eid Aqras ($130 \pm 4.58 \mu\text{gkg}^{-1}$) and AL-Brazeq ($167 \pm 3.78 \mu\text{gkg}^{-1}$) where the dough is fermented by yeast for a period of two hours and one hour, respectively.

Table 2: Acrylamide content (μgkg^{-1}) of traditional foods

Traditional Food	AA Median ($\mu\text{g kg}^{-1}$)	No. of samples
Eid Aqras	130 ± 4.58^a	2
Brazeq	167 ± 3.78^b	2
Baqlawwa	172 ± 4.35^b	2
Fatayr	192 ± 3.51^c	2
Fried bread	230 ± 4.06^d	2
Kenafa	242 ± 2.64^e	2
AL-namora	282 ± 4.35^f	2
Awamat	421 ± 2.64^g	2
Mshabak	481 ± 2.08^h	2

Note: The results are presented as mean \pm standard deviation (SD) ($n = 3$); a–h values are significantly different from each other and significance accepted at $p \leq 0.05$

DISCUSSION

This study focused on Syrian foods available in Syria, such as common commercial food products and some traditional foods. Wheat is the most important staple food commodity in Syria and is consumed primarily as bread, which is mainly composed of wheat flour, salt and water, and leavened using yeast. For the determination of the AA content in wheat products, widely consumed bread types were selected including white wheat bread, whole wheat bread, soft bread, and bread sticks.

The data showed that the AA content in whole wheat bread was significantly higher $p < 0.05$ than AA in bread made with white flour, with the results of $153 \pm 2.64 \mu\text{gkg}^{-1}$ for Syrian whole wheat bread and $119 \pm 1.7 \mu\text{gkg}^{-1}$ for Syrian white flour bread. This may be due to the difference in AA precursors in bread such as asparagine (Asn) and reducing sugars since whole wheat flour has higher levels of Asn and reducing sugars than white flour [20] thereby leading to more AA formation in these products.

Levels of AA greater than $1000 \mu\text{gkg}^{-1}$ were found in biscuit-related products. The AA concentration range of these products was rather wide ($1433 \pm 2.51 - 57 \pm 2.64 \mu\text{gkg}^{-1}$), which could be attributed to differences in the recipes and technology, baking temperature and time, and reducing sugars and Asn content. It was demonstrated that ingredients such as almonds and sesame may impact on the AA concentration of bakery products [21].

A previous study showed that the fermentation by yeast decreases the free Asn in raw dough, which resulted in less formation of AA during the baking process; furthermore, prolonged fermentation can lower the AA content by up to 87 % [28]. On the other hand, the highest levels of AA were found in AL-Awamat and AL-Mshabak ($421 \pm 2.64 - 481 \pm 2.08 \mu\text{gkg}^{-1}$), which are considered to be commonly consumed local fried sweets in Syria, resembling doughnuts; they are leavened by yeast for 10 min and deep fried.

Al-Mshabak dough is prepared from coarse semolina flour while AL-Awamat dough is made from white wheat flour. It was also shown that the AA content in AL-Kenafa ($242 \pm 2.64 \mu\text{gkg}^{-1}$) was lower than in AL-Namora ($282 \pm 4.35 \mu\text{gkg}^{-1}$) and this could also be related to the differences in the heat treatment, as AL-Kenafah is baked at a lower temperature of $180 - 200 \text{ }^\circ\text{C}$, for a shorter time of 10 min, while the baking temperature and period for AL-Namora is about $200 - 250 \text{ }^\circ\text{C}$ for 25 min.

These differences in product composition and formula are a major factor accounting for differences in the levels of AA precursors; additionally, differences in leavening agents and varieties of processing conditions all play a contributing factor in AA formation in food

This study also showed that the AA content in bakery products that are made with corn flour was lower than those made with wheat flour. These results may support the concept that AA is

formed less in corn products than in wheat products [22]. These data were in accordance with those found in previous studies, in which Rufian-Henares *et al* detected AA levels in commercial biscuits and bread types marketed in Spain and found an AA concentration range of $30 - 2085 \mu\text{gkg}^{-1}$ for biscuits, $30 - 151 \mu\text{gkg}^{-1}$ for crisp bread, $30 - 296 \mu\text{gkg}^{-1}$ for crackers and $30 - 323 \mu\text{gkg}^{-1}$ for bread sticks [15]. In another study, AA levels in breakfast cereals were $62 - 803 \mu\text{gkg}^{-1}$ [23].

In this study some popular Syrian potato and corn products that are cooked at high temperatures were analyzed to detect toxic AA content, which was reported for the very first time.

The variety of AA levels in the same product samples (396 and $1844 \mu\text{gkg}^{-1}$ in potato products) may be attributable to the various cooking conditions such as temperature, duration of frying, sort of frying oil and the variety of potatoes used, which may contain different amounts of reducing sugars. There are reports suggesting that AA content in food products is affected by different factors relating to differences in the methods of production such as type of frying oil, frying time and temperature [24].

Furthermore the data showed that potato chips contain AA concentrations significantly higher $p \leq 0.05$ than corn chips (minimum $396 \mu\text{gkg}^{-1}$ for potato products compared to $183 \mu\text{gkg}^{-1}$ for corn, and a maximum of $1844 \mu\text{gkg}^{-1}$ compared to $366 \mu\text{gkg}^{-1}$) and this may be due to the potato containing more reduced sugar and Asn amino acid than corn and other cereals. Figure 1 shows AA acrylamide levels in corn and potato chips. Numerous studies have been carried out for AA levels in potato products in different countries. In Iran, the study of AA content in Iranian brands of potato and corn products revealed that the AA levels ranged between $244 - 1688 \mu\text{gkg}^{-1}$ in potato products and between $30 - 410 \mu\text{gkg}^{-1}$ in corn products [25]. In Austria, the maximum levels of AA in potato crisps was $1500 \mu\text{gkg}^{-1}$ [26], while in Poland the amount of AA in potato crisps was between $352 - 3647 \mu\text{gkg}^{-1}$ depending on the type of crisps [16]. In Japan, AA concentrations in most potato chips and whole potato-based fried snacks were found to be greater than $1000 \mu\text{gkg}^{-1}$ [11].

Like bakery and potato products, different brands of ground coffee were analyzed. The study showed that the AA content varies in coffee powder samples. However, this could be attributed to the differences in the coffee beans

used for blend ground coffee and also differences in the heat treatments applied during the roasting process with respect to the fact that these parameters are known to play an important role in AA generation in coffee. It was also found that dark roasted coffee powder contained an AA content lower than that of medium roasted coffee meaning that, by virtue of the increase in roasting period and temperatures, there could have been more AA elimination/degradation than AA formation. It has been reported that AA is generated at the beginning of the roasting step and, when it reaches the maximum level, it starts to slowly decrease [27].

CONCLUSION

This is the first study concerning the determination of AA content in traditional and common commercial foods in Syria. The study's results provided useful information to add to the AA food database; they also play vital role in the assessment of AA exposure among the Syrian population. These data are almost comparable with studies conducted on AA content originating from different countries e.g., in Asia, the United States, and Europe. In addition, the results agree with previous reports, which indicate that potato products contain a higher content of AA than corn products, and also confirms that AA concentrations vary according to the food ingredients and the processing conditions.

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DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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