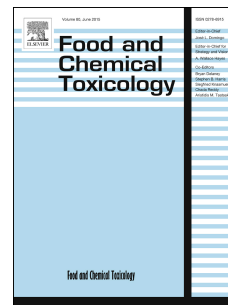


Accepted Manuscript

Health risk assessment of acrylamide in bread in Iran using LC-MS/MS

Samira Eslamizad, Farzad Kobarfard, Christina Tsitsimpikou, Aristides M. Tsatsakis, Kimia Tabib, Hassan Yazdanpanah



PII: S0278-6915(19)30072-9

DOI: <https://doi.org/10.1016/j.fct.2019.02.019>

Reference: FCT 10349

To appear in: *Food and Chemical Toxicology*

Received Date: 31 December 2018

Revised Date: 6 February 2019

Accepted Date: 8 February 2019

Please cite this article as: Eslamizad, S., Kobarfard, F., Tsitsimpikou, C., Tsatsakis, A.M., Tabib, K., Yazdanpanah, H., Health risk assessment of acrylamide in bread in Iran using LC-MS/MS, *Food and Chemical Toxicology* (2019), doi: <https://doi.org/10.1016/j.fct.2019.02.019>.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Health risk assessment of acrylamide in bread in Iran using LC-MS/MS

Authors: Eslamizad Samira^a, Kobarfard Farzad^{a,b,c}, Christina Tsitsimpikou^d, Aristides M. Tsatsakis^e,
Tabib Kimia^c, Yazdanpanah Hassan^{a,f,*}

^a Food Safety Research Center, Shahid Beheshti University of Medical Sciences, Tehran, IR Iran.

^b Phytochemistry Research Center, Shahid Beheshti University of Medical Sciences, Tehran, IR Iran.

^c Department of Medicinal Chemistry, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, IR Iran.

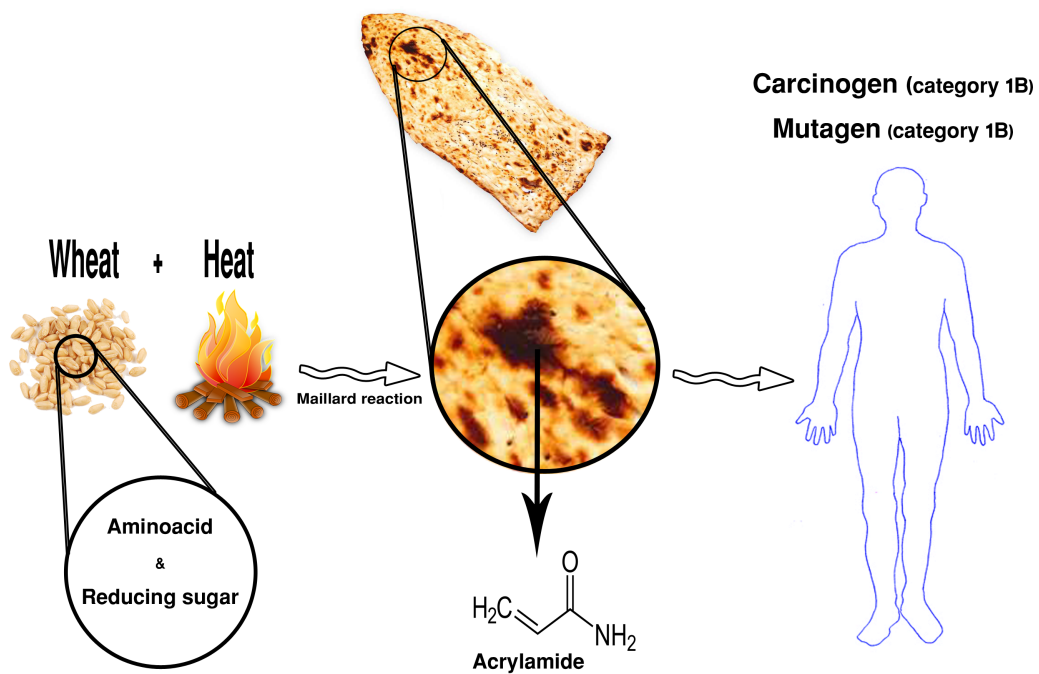
^d General Chemical State Laboratory of Greece, 11521 Athens.

^e Center of Toxicology Science & Research, Medical School, University of Crete, Heraklion, Crete, Greece.

^f Department of Toxicology and Pharmacology, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, IR Iran.

Corresponding author: Hassan Yazdanpanah, Food Safety Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran, and Department of Toxicology and Pharmacology, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, IR Iran.

E-mail: yazdanpanah@sbmu.ac.ir, Tel/Fax: +98 21 88 200080/+98 2188209620



Health risk assessment of acrylamide in bread in Iran using LC-MS/MS**Abstract:**

Acrylamide is a chemical, often present in bread, legally classified as carcinogen, mutagen and reproductive toxicant. Since bread is consumed both world-wide and in Iran, determination of acrylamide in different types of breads is of high interest. In the present study, acrylamide was monitored in 56 Sangak and 30 industrial bread samples collected from Tehran and Shiraz, using LC-MS/MS (LOQ=1 ng/g). In addition, the noncarcinogenic risk (target hazard quotient–THQ) and carcinogenic risk (incremental lifetime cancer risk–ILCR) due to ingestion of acrylamide through bread consumption in children and adults were assessed. Acrylamide was detected in more than 90% of the samples tested. The average daily intake of acrylamide in Iran based on exclusive consumption of Sangak bread, was estimated at 145 ng/kg bw/day. Based on the THQ for bread acrylamide in adults and children, the decreasing risk order was: Shiraz semi-industrial Sangak, Shiraz traditional Sangak, Tehran traditional Sangak, Tehran industrial bread. The ILCR of bread acrylamide calculated for adults and children was higher than the permissible lifetime carcinogenic risk value established by USEPA ($1.00E-5$). Results show that bread is a major source of acrylamide intake by people in Iran and all consumers regardless of age could be at elevated carcinogenic risk.

Key words: Risk assessment, Acrylamide, Bread, LC-MS/MS, and Iran.

1. 1. Introduction

Acrylamide is a chemical process contaminant found in foods as a byproduct of the cooking process. More specifically, when the cooking temperature exceeds 120°C, such as in baking, grilling, frying and toasting, the amino acid asparagine (found in certain foods) can react with reducing sugars via the Maillard reaction to produce acrylamide (Mottram, Wedzicha, & Dodson, 2002; Stadler et al., 2002; Tareke, Rydberg, Karlsson, Eriksson, & Törnqvist, 2002).

Rivadeneira-Dominguez et al. (2018) reported that systemic administration of acrylamide to Wistar rats causes deleterious effects on renal and hepatic function, producing dose-dependent alterations of blood chemistry and cytometry parameters (Rivadeneira-Dominguez, Becerra-contreras, & Vazquez-luna, 2018). Acrylamide has a harmonized classification in the European Union as carcinogen (category 1B), mutagen (category 1B) and reproductive toxicant (category 2, fertility) (EC Regulation 1272/2008 of The European Parliament and of Council, 2013) and these hazardous effects of acrylamide are recognized world-wide (Anne McDonald, 1995; European commission, 2002).

For a long time, bread has been a basic part of human diet all over the world (Birch, Petersen, & Hansen, 2014; Pico, Bernal, & Gómez, 2015). It represents a good source of energy, protein, dietary fiber (DF), minerals, vitamins and many alternative bioactive compounds (Dastmalchi, Razavi, Faraji, & Labbafi, 2016). In Iran, with more than 80 million inhabitants, there are three types of bread available in the market: traditional, industrial and semi-industrial. One of the most popular traditional breads in Iran is Sangak which is made from whole wheat flour. Hamburger buns are another popular type of bread which are considered industrial bread, made with white wheat flour (Dastmalchi et al., 2016). Based on the national nutrition survey, the mean bread ingestion rate in Iran for urban and rural population has been estimated to be 320 g per person

per day or 116 kg per person per year (National Nutrition and Food Technology Research Institute, 2004) which is considerably higher compared to bread consumption in European countries (Statista, 2013). The respective value for urban population is reported lower (286 g per person per day) (National Nutrition and Food Technology Research Institute, 2004).

Numerous methods for quantification of acrylamide in food have been reported (Evrin, Tekkeli, & Önal, 2012). High performance liquid chromatography (HPLC) coupled with mass spectrometry (MS) are the most preferred methods for the separation and quantification of acrylamide residues in foods, due to their sensitivity, selectivity and versatility (Hu, Xu, Fu, & Li, 2015).

Considering the carcinogenic and genotoxic effects of acrylamide on one hand and the high consumption of bread in Iran on the other hand, determination of this contaminant in breads consumed in Iran and the respective health risk characterization and finally assessment becomes necessary. Risk assessment is a process which includes several steps: hazard assessment (hazard identification and hazard evaluation), exposure assessment and risk characterisation.

Therefore, the aim of this study was to determine the levels of acrylamide in different types of Sangak bread commonly consumed in Iran and hamburger buns, using LC/MS/MS combined with an easy, cheap and safe extraction and clean-up method. The results will be used in the exposure assessment and characterization of the relevant risks.

2. Materials and Methods

2.1. Sample collection

Nine traditional and sixteen semi-industrial bread samples were collected from Sangak bakeries located in Shiraz city. Shiraz is located in the south west of Iran, with a population of around

1,900,000 and about 90 Sangak bakeries operating. Thirty industrial and 31 traditional bread samples were collected from Sangak bakeries and retail stores located in Tehran City (Tehran is the most populous city and capital of Iran. The population of Tehran is about 13,000,000 in 2017, with about 550 Sangak bakeries, respectively. All bread samples had been baked from wheat flour. After collection, all samples were covered with aluminum foil to prevent photodegradation and transported to the lab. Each sample was separately cut into small pieces and blended using a blender. The samples were kept in amber glass bottles with Teflon-lined caps at -20°C until analysis.

2.2. Chemicals

Acrylamide and acrylamide-d₃ as internal standard were purchased from Sigma Aldrich (St. Louis, Mo., USA), HPLC grade solvents including acetonitrile, methanol, and acetone were purchased from Merck (Darmstadt, Germany). Zinc sulfate and potassium hexacyanoferrate were obtained from Chem Lab NV, Belgium. Primary Secondary Amine (PSA) SPE Bulk Sorbent was obtained from Varian, Italy. A water purification system was used for the preparation of ultrapure water (Econolab, Oklahoma, USA).

2.3. Preparation of standards and reagents

Acrylamide and acrylamide-d₃ stock solutions were prepared at 1 mg/ml concentration in distilled water. Intermediate standard solutions of acrylamide contained concentrations of 100 mcg/ml and 10 mcg/ml in distilled water. An intermediate standard solution for acrylamide-d₃ contained 10,000 ng/ml in water. Bread samples were spiked with acrylamide at 20-3000 ng/ml.

Fifty μL of each working standard solution was added to 1 g of blank bread samples. A total of 100 μL of acrylamide-d₃ solution was added to each of the spiked bread sample.

To avoid light exposure, all standard solutions were prepared in an amber colored, volumetric flask and stored at 4°C until required. The samples so obtained were treated as described in the sample preparation section. Carrez I solution was prepared by dissolving 1.5 g of potassium hexacyanoferrate in 10 ml of water and CarrezII solution by dissolving 3 g of zinc sulfate in 10 ml of water.

2.4. Sample preparation

The extraction procedure is as follows: 1 g sample was weighed into a 15 ml centrifuge tube, and 100 μL of 10,000 ng/ml of the acrylamide-d₃ solution and 2.5 ml methanol were added. The tube was shaken with a vortex shaker for 20 s, followed by centrifugation of the mixture at 5000 RPM for 10 min. The whole methanol extract was transferred to a 15 ml centrifuge tube and then 50 μl of Carrez I and II solutions were added. The tube was shaken using a vortex shaker for 11 s. Fifty mg PSA was added to the tube and shaken for 10 s. Thereafter, the mixture was centrifuged at 5000 RPM for 10 min. The whole methanol extract was transferred to a 2 ml microtube. The extract was evaporated under the gentle flow of nitrogen gas until remaining about 100-150 μl of the extract. The remaining extract was dissolved in 500 μL of water, followed by shaking for 10 s. Finally, 400 μl of the extract was transferred to an amber vial and 70 μl injected to LC-MS/MS.

2.5. Liquid Chromatography—Mass Spectrometry Condition

The quantification of acrylamide was performed by an Agilent 1200 model HPLC system (Agilent Santa Clara, CA, USA) consisting of a binary pump, an Autosampler and a temperature controlled column oven, coupled to an Agilent 6410 Triple Quadrupole mass spectrometer system equipped with electrospray ionization (ESI) interface.

The analytical separation was performed on an ODS-H optimal-C18, Capital (150mm × 4.6 mm, 3µm) column using an isocratic mixture of 0.1% acetic acid in an aqueous solution of formic acid and 3% methanol (97:3, v/v) at a flow rate of 0.5 ml/min.

The electrospray was operated in the positive ion mode with the capillary set at 4.0KV and the collision energy at 10 eV. The source gas temperature was set at 325°C and the desolvation temperature was set at 400°C. Nitrogen was used as a nebulizer gas (flow 10L/min) and desolvation gas (flow 150 L/h).

The multiple reaction monitoring (MRM) mode of the degradation patterns m/z 72 → 55 (acrylamide) and m/z 75 → 58 (Acrylamide-d3) were used for quantification.

2.6. Method validation

For method validation, the parameters assessed were linearity, limit of detection (LOD), limit of quantification (LOQ), recovery, precision and uncertainty. In order to overcome the matrix effect, the calibration curve was drawn with spiked samples in the presence of an internal standard (d3-Acrylamide).

The linearity of the method for the analysis of acrylamide was evaluated by building the spiked calibration curves over the acrylamide concentration range of 1–150 ng/g (1, 2.5, 5, 10, 30, 100 and 150 ng/g) and then treated according to the previously described procedure. The calibration curve for acrylamide analysis was constructed by plotting the ratio peak area of acrylamide to

peak area of d3-acrylamide versus the spiked concentration levels of acrylamide. An internal standard method was used for quantification of the acrylamide in bread samples.

2.7. Quality control samples (QC)

Four quality control, bread samples spiked at the level of 30 ng/g were analyzed. The average recovery and RSD (%) of QC samples were 90.42% and 6.63%, respectively.

2.8. Risk assessment

2.8.1. Non-carcinogenic risk

The two determinative factors in Chronic Daily Intake (CDI) are the acrylamide concentration in food and the daily food consumption; however, the body weight may affect tolerance. The CDI was separately calculated for the adults and children population according to the following equation:

$$CDI = \frac{C \times IRi \times EDi \times EFi}{BW \times AT} \quad (1)$$

where CDI is the chronic daily intake (mg/kg/day); EFi is exposure frequency (365 days/year), EDi is the exposure duration (70 years for adults and 6 years for children), IRi is the ingestion rate (320 g/person/day in Iran) (National Nutrition and Food Technology Research, 2004), C is the acrylamide concentration in bread (mg/g), BW is body weight (average BW for the adults and children were considered 70 and 15 kg, respectively, according to EPA recommendations) (Shahrbabki et al., 2018) and AT is the average exposure time for non-carcinogens (365 days/year EDi).

For the determination of the potential health risks for hazard classes other than carcinogenicity, the non-cancer risk characterization methodology of Target Hazard Quotient (THQ) was also used (Storelli, 2008).

The non-carcinogenic risk for the consumers of the selected bread types tested in the present study was estimated by Equation 2 (Shahrbabki et al., 2018):

$$THQ = \frac{CDI}{RfD} \quad (2)$$

where THQ is the target hazard quotient; CDI is chronic daily intake, RfD is the oral reference dose (mg/kg/day), which does not differ between adults and children (IRIS, 2012). For acrylamide RfD is 0.002 (mg/kg BW/day) (IRIS, 2012). When THQ is higher than 1, the population is at considerable non-carcinogenic risk (Shahrbabki et al., 2018).

2.8.2. Estimation of carcinogenic risk

The carcinogenic risk for the consumers due to consumption of the tested bread types was estimated using Incremental Lifetime Cancer Risk (ILCR) and calculated by Equation (3) (Shahrbabki et al., 2018):

$$ILCR = CDI \times CSF \quad (3)$$

where, CDI is the chronic daily intake (mg/kg/day); CSF is Cancer Slope Factor (mg/kg per day) as the risk caused by lifetime averages dose of 1 mg/kg BW/day (Shahrbabki et al., 2018). CSF for acrylamide is 0.5 (mg/kg per day) (IRIS, 2012).

3. Results

3.1. Method validation

Results showed that the calibration curve of acrylamide was linear in the range of 1–150 ng/g, with the correlation coefficient of $R^2 = 0.999$ (Figure 1).

The extraction recoveries were determined by applying the full procedure to triplicate samples in three days at three spiking levels including 1.5, 30 and 50 ng/g. Appropriate recoveries (90.0–101%) of acrylamide from spiked samples were obtained. The values obtained for CV% were less than 10.3%. Both the recoveries and CV were in accordance with the criteria set by European guidelines (SANTE, 2017) (Table 1). The developed method is very sensitive and the LOD and LOQ were 0.3 and 1 ng/g, respectively. The expanded measurement uncertainty, was calculated using a coverage factor of 2 which gives a level of confidence of approximately 95% ($U = 2u$) (Table 1) (European commission, 2015).

Please insert figure 1 and table 1

3.2. Determination of acrylamide in bread samples

The results of acrylamide in bread samples are presented in Table 2 and Figure 2.

The results indicate that in the vast majority (>82%) the samples tested, acrylamide residues were detected in levels > LOQ. More specifically, only in 1 out of the nine traditional bread samples collected from Shiraz no acrylamide was detected. Similarly, in 4 out of the 30 industrial bread samples collected from Tehran acrylamide levels were below LOQ. The mean concentration of acrylamide in traditional Sangak of Shiraz was 2 times higher than traditional Sangak of Tehran and the mean concentration of acrylamide in semi-industrial Sangak of Shiraz was 2.2 times higher than traditional Sangak of Tehran. The mean concentration of acrylamide in

semi-industrial Sangak of Shiraz, traditional Sangak of Shiraz and traditional Sangak of Tehran was 6, 5.4 and 2.7 times higher than industrial bread of Tehran, respectively.

Please insert table 2 and figure 2

3.3. Dietary exposure

To estimate the exposures to acrylamide associated with the consumption of traditional, semi-industrial and industrial bread samples, it is essential to determine the dietary intake by the Iranian population. The calculations are shown in Table 3.

Please insert table 3

Dietary exposure to acrylamide for people from Shiraz via consumption of semi-industrial Sangak and traditional Sangak bread was 2 and 2.2 times higher than dietary exposure to acrylamide in people from Tehran via consumption of traditional Sangak. Dietary exposure to acrylamide for people from Tehran via consumption of industrial bread was about 6 times less than people from Shiraz via consumption of semi-industrial Sangak bread. The general population's dietary exposure to acrylamide in Shiraz only through Sangak bread, assuming a person consumed 50% of traditional Sangak bread and 50% semi-industrial Sangak bread, was estimated to be 190ng/kg bw/day.

The daily intake of acrylamide in Tehran's population through traditional and industrial bread consumption, assuming a person consumed 50% of traditional Sangak bread and 50% industrial Sangak bread was estimated to be 60.5 ng/kg bw/day. Since there is no available data for bread consumption by children in Iran, no specific calculations for children were made.

3.4. Risk assessment

3.4.1. Non-carcinogenic risk

The calculated CDI and THQ of acrylamide for Iranian population via consumption of the selected bread are presented in Table 4a.

The CDI for acrylamide in selected bread in adult and children are in the following decreasing risk order: Shiraz semi-industrial Sangak > Shiraz traditional Sangak > Tehran traditional Sangak > Tehran industrial.

The THQ is a valuable parameter for assessing the risks associated with consumption of selected product contaminated with acrylamide. The THQ assessments for acrylamide risk for Iranian population posed by bread consumption are shown in Table 4a. THQ was calculated in the following decreasing order: children in Shiraz consuming semi-industrial Sangak > children in Shiraz consuming traditional Sangak > children in Tehran consuming traditional Sangak > adults in Shiraz consuming semi-industrial Sangak > adults in Shiraz consuming traditional Sangak > children in Tehran consuming industrial bread > adults in Tehran that consuming traditional Sangak > adults in Tehran that consuming industrial bread. As shown in Table 4a, the calculated THQ for adults and children in Shiraz consuming semi-industrial sangak bread was about 6 times higher than adults and children in Tehran consuming industrial bread. The calculated THQ for adults and children in Shiraz consuming traditional sangak bread was 2 times higher than adults and children in Tehran that consuming the same bread.

Please insert table 4a

3.4.2. Estimation of carcinogenic risk

The permissible lifetime carcinogenic risk established by USEPA is $1.00E-5$ (risk of developing cancer is 1 in 100,000 exposed population) (IRIS, 2012). The ILCR assessments of acrylamide for Iranian adults and children posed by the selected bread consumption are

shown in Table 4b. ILCR was calculated in the following decreasing order: children in Shiraz that consuming semi-industrial Sangak > children in Shiraz that consuming traditional Sangak > children in Tehran that consuming traditional Sangak > adults in Shiraz that consuming semi-industrial Sangak > adults in Shiraz that consuming traditional Sangak > children in Tehran that consuming industrial > adults in Tehran that consuming traditional Sangak > adults in Tehran that consuming industrial. The results show that ILCR for adults and children in Shiraz due to consumption of semi-industrial Sangak bread is 5 times higher than that for people in Tehran who consume industrial bread. And also ILCR for adults and children in Shiraz and Tehran due to consumption of traditional Sangak bread, is about 5.5 and 2.7 times higher than people in Tehran that consume industrial bread, respectively.

4. Discussion

4.1. Occurrence of acrylamide in bread

Several studies have been conducted on the contamination of bread with acrylamide. On the basis of the last JECFA evaluation about acrylamide occurrence, data from more than 12500 samples reported from 31 countries, reveal that national mean concentrations of acrylamide in crisp breads and crackers ranged from 87 to 459 $\mu\text{g}/\text{kg}$ (FAO/WHO, 2010). Mean concentration of acrylamide in our results was less than national mean concentrations of acrylamide in crisp breads and crackers in JECFA evaluation. On the basis of the EFSA report (EFSA, 2015), the mean concentration of acrylamide in 302 wheat soft bread samples was 38 $\mu\text{g}/\text{kg}$ which was about 5 times higher than acrylamide mean concentration in industrial bread in our study.

In a survey carried out in Poland, the mean value of acrylamide levels in Crisp bread was 430 $\mu\text{g}/\text{kg}$ that was much higher than our results (Mojska, Gielecinska, Szponar, & Oltarzewski,

2010). In Finland, (Hirvonen et al., 2011) the highest acrylamide levels in Crisp bread (674 $\mu\text{g}/\text{kg}$), followed by potato crisps (539 $\mu\text{g}/\text{kg}$) and sweet biscuits (443 $\mu\text{g}/\text{kg}$). The highest acrylamide level in Crisp bread was much higher than (6.5 times) maximum range of acrylamide concentration in our results.

In France, the acrylamide levels in bread and bread products were found around 34 $\mu\text{g}/\text{kg}$, which means that the level of acrylamide in French breads was slightly higher than the mean concentration of acrylamide in tested bread in our study (Sirot, Hommet, Tard, & Leblanc, 2012).

The mean concentration of acrylamide in bread samples in Iran was lower than samples in some other countries including Germany (crispbread: 916 $\mu\text{g}/\text{kg}$), Portugal (whole grain bread: about 787 $\mu\text{g}/\text{kg}$), Sweden (crispbread: about 300 $\mu\text{g}/\text{kg}$) and Spain (crispbread: about 100 $\mu\text{g}/\text{kg}$). (EFSA, 2015; Hilbig, Freidank, Kersting, Wilhelm, & Wittsiepe, 2004; Jesus et al., 2018; Mustafa, 2008; Iveta Pugajeva, Zumbure, Melngaile, & Bartkevics, 2014; Taylor, Rufianhenares, Arribas-lorenzo, & Morales, 2007) .

However, the concentration of acrylamide in bread samples studied in the present work was about 2 times higher than samples in Latvia (14 $\mu\text{g}/\text{kg}$) and almost equal concentration of acrylamide in bread in some countries such as Turkey (28.97 $\mu\text{g}/\text{kg}$), Korea (33.0 ± 4.9 $\mu\text{g}/\text{kg}$) and China (About 35 $\mu\text{g}/\text{kg}$) (Alpozen, Guven, Ozdestan, & Uren, 2015; Kim, Hwang, & Lee, 2007; I Pugajeva, Zumbure, L, & 2014, 2014; Zhuang, Zhang, Liu, & Yuan, 2012).

The acrylamide levels in tested breads were lower than the indicative acrylamide value of wheat based bread (80 $\mu\text{g}/\text{kg}$) (European commission, 2013).

There are a few reports about the amount of acrylamide in bread in Iran (Table 6). Sadeghi et al. (Sadeghi et al., 2016) evaluated acrylamide levels in 48 various traditional and industrial types of

bread using LC-MS/MS. According to their results, the increasing acrylamide level order in the studied bread was averagely as follows: French baguette, Barbari, hamburger buns, wholegrain baguette, breadcrumbs, Sangak, Taftoon (a leavened flour bread), and lavash (a soft, thin unleavened flatbread). This amount of acrylamide in tested bread was about 3 times of acrylamide concentration in our tested bread that are presented in table 6. Dastmalchi et al (Dastmalchi et al., 2016) reported the mean concentration of 135 $\mu\text{g}/\text{kg}$ for acrylamide in Sangak bread which was about 3 times higher than the mean concentration of acrylamide in traditional Sangak bread in our study (as shown in table 6). Motaghi and co-workers (Motaghi, Seyedain Ardebili, Honarvar, Mehrabani, & Baghizadeh, 2012) studied acrylamide of Sangak bread samples by GC-FID. The result was about 2 times higher than our result. Tables 5 and 6 present a summary of data on acrylamide concentration in various countries and Iran.

Please insert tables 5 and 6

4.2. Dietary exposure

The estimates of dietary exposure on the basis of the JECFA report indicated that the major foods contributing to dietary exposure were potato products (crisps and French fries), breads and rolls, as well as pastry and sweet biscuits (cookies) (FAO/WHO, 2010). Unfortunately, in Iran data on the contribution of other foods in acrylamide dietary exposure is not available.

In the European Prospective Investigation into Cancer and Nutrition (EPIC) study, Freisling et al. in 2013 estimated the acrylamide intake in 27 centres of 10 European countries. A total of 39 994 participants, aged 35–74, completed a standardized 24-hour dietary recall. The mean intake (minimally adjusted by gender) across centres ranged from 12 to 41 μg per day for women and from 15 to 48 for men. The main contributors to the intake were the food groups ‘bread, crisp bread, rusks’, followed by ‘coffee’ and ‘potatoes’. It was observed that intakes were higher in

northern European countries (EFSA, 2015). In Finland, Hirvonen et al. (2011) estimated the dietary acrylamide exposure of Finnish adults combining the Finnish occurrence data reported in the literature and the food consumption data from the FINDIET 2007 survey (2 038 adult participants, 25–44 years old). The estimated median (97.5th percentile) exposure for adults was 0.44 (1.16) $\mu\text{g}/\text{kg}$ B.w. per day for women, and 0.41 (0.87) $\mu\text{g}/\text{kg}$ B.w. per day for men. The main contributor to the acrylamide intake was ‘coffee’ followed by ‘starch-rich casserole’, ‘rye bread’ and ‘biscuits’ (Hirvonen et al., 2011).

There are few data relating to the exposure of acrylamide through different sources of foods, hence its intake was estimated via only tested breads.

A comparison of the results of this study with JECFA results about acrylamide dietary exposure (1 $\mu\text{g}/\text{kg}$ b.w. per day at the mean, and 4 $\mu\text{g}/\text{kg}$ b.w. per day for a consumer at a high percentile of the distribution) (FAO/WHO, 2010) showed that the highest acrylamide intake could be attributed to the consumption of semi-industrial Shiraz Sangak bread that is about 25% of the mean acrylamide dietary exposure. The average intake of acrylamide through 4 types of bread tested is about 12.5% of the mean acrylamide dietary exposure reported by JECFA.

In real life, consumers are exposed to complex mixtures of chemicals through consumption, water, common commercial products, cosmetics and personal care products. Apart from acrylamide, chemicals in trace quantities could be released from food packaging and other food contact materials, and in combination with other compounds generated during food processing, could constitute a clustering risk for public health (Docea et al., 2018; Tsatsakis et al., 2017). In addition, combinations of toxic stimuli could produce damage even when the exposure level of each chemical in the mixture is less than the lowest adverse effect level or the regulatory limit

for the said chemical (Kostoff, Goumenou, & Tsatsakis, 2018). Since risk assessment so far focuses on individual compounds in a commercial product, food included, it doesn't assess the overall risk of mixture of chemicals through all sources of exposures.

4.3. Risk assessment

Based on the results of present studies it could be speculated that children in Shiraz that consume semi-industrial sangak bread have the highest potential health risk of acrylamide intake through bread. Indeed the children that consume traditional Sangak of Shiraz and traditional Sangak from Tehran are at the high potential health risk. Even though, all values are below 1 which indicates no risk but acrylamide could be taken from others foods and also other sources and therefore these calculated THQ for each group, only through consumption of the selected bread, seems to be high. Therefore, exposure of acrylamide through bread and other sources and also combinations of other compounds might have the potential to induce adverse health effects.

Results of our study indicate that Incremental Lifetime Cancer Risk (ILCR) of acrylamide for adult and children due to ingestion of the breads are higher than $1.00E-5$ value. Therefore, all age groups consumers are at elevated exposure risk. However a majority of the studies reported no statistically significant association between dietary acrylamide intake and various cancers, and few studies reported increased risk for renal, endometrial, and ovarian cancer (Virk-Baker, Nagy, Barnes, & Groopman, 2014). In addition, there is no clear epidemiological evidence that acrylamide at low doses actually increases cancer risk significantly and reference doses represent the upper level of confidence of low risk. On the other hand, the lack of clear associations between dietary acrylamide exposure and cancer does not of course mean that there is no link at all. The decreasing order of age groups based on ILCR

is: children (ingestion of Shiraz semi-industrial Sangak), children (ingestion of Shiraz traditional Sangak), children (ingestion of Tehran traditional Sangak), adults (ingestion of Shiraz semi-industrial Sangak), adults (ingestion of Shiraz traditional Sangak), children (ingestion of Tehran industrial bread), adults (ingestion of Tehran traditional Sangak), adults (ingestion of Tehran industrial bread).

5. Conclusions

In this study, a sensitive method was used for determination of acrylamide in bread samples. The results revealed that acrylamide in traditional and semi-industrial bread samples are higher than industrial samples, andk bread could be a major source for acrylamide intake by people in Tehran and Shiraz. Although, all calculated THQ are below 1, which indicates controlled risk, exposure of acrylamide through bread and other sources and also combinations of other compounds might have the potential to induce adverse health effects. The calculated results showed ILCR of acrylamide for adult and children due to bread ingestion were higher than permissible lifetime carcinogenic risk value established by USEPA ($1.00E-5$), and all age groups consumers are at elevated carcinogenic risk. But, it should be noted that there is no clear epidemiological evidence that acrylamide at low doses actually increases cancer risk significantly and some foods containing elevated levels such as bread are associated with reduced cancer risk.

Considering the real-life exposure scenario in which an individual is exposed to mixtures of chemicals from different sources (environment, foodstuff, lifestyle products and water consumption), even if acrylamide is found at very low concentrations, long-term exposure of combinations of other compounds might have the potential to induce adverse health effects.

Therefore, further studies are necessary for the monitoring acrylamide in different food products as well as estimating average dietary exposure and health risk assessment of acrylamide for main foods in Iran.

Acknowledgments

The authors are very grateful to the Iran National Science Foundation of Presidential Office for their support.

References

- Adel, M., Oliveri Conti, G., Dadar, M., Mahjoub, M., Copat, C., & Ferrante, M. (2016). Heavy metal concentrations in edible muscle of whitecheek shark, *Carcharhinus dussumieri* (elasmobranchii, chondrichthyes) from the Persian Gulf: A food safety issue. *Food and Chemical Toxicology*, *97*, 135–140. <https://doi.org/10.1016/j.fct.2016.09.002>
- Alpozen, E., Guven, G., Ozdestan, O., & Uren, A. (2015). Determination of acrylamide in three different bread types by an in-house validated LC-MS/MS method. *Acta Alimentaria*, *44*(2), 211–220. <https://doi.org/10.1556/AAlim.2013.3333>
- Alyousef, H. A., Wang, H., Al-Hajj, N. Q. M., & Koko, M. Y. F. (2016). Determination of acrylamide levels in selected commercial and traditional foods in Syria. *Tropical Journal of Pharmaceutical Research*, *15*(6), 1275. <https://doi.org/10.4314/tjpr.v15i6.21>
- Anne McDonald. (1995). Some Industrial Chemicals: IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Vol 60. *Occup Environ Med*, *52*(5), 360. Retrieved from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1128232/%7B#%7D>
- Bermudo, E., Moyano, E., Puignou, L., & Galceran, M. T. (2008). Liquid chromatography coupled to tandem mass spectrometry for the analysis of acrylamide in typical Spanish products. *Talanta*, *76*(2), 389–394. <https://doi.org/10.1016/J.TALANTA.2008.03.011>
- Birch, A. N., Petersen, M. A., & Hansen, Å. S. (2014). Aroma of wheat bread crumb. *Cereal Chemistry*. Wiley-Blackwell. <https://doi.org/10.1094/CCHEM-06-13-0121-RW>
- Dastmalchi, F., Razavi, S. H., Faraji, M., & Labbafi, M. (2016). Effect of *Lactobacillus casei*-

- casei and *Lactobacillus reuteri* on acrylamide formation in flat bread and Bread roll. *Journal of Food Science and Technology*, 53(3), 1531–1539. <https://doi.org/10.1007/s13197-015-2118-3>
- Docea, A. O., Gofita, E., Goumenou, M., Calina, D., Rogoveanu, O., Varut, M., ... Tsatsakis, A. (2018). Six months exposure to a real life mixture of 13 chemicals' below individual NOAELs induced non monotonic sex-dependent biochemical and redox status changes in rats. *Food and Chemical Toxicology*, 115(March), 470–481. <https://doi.org/10.1016/j.fct.2018.03.052>
- EC Regulation 1272/2008 Of The European Parliament And of Council. (2013). Regulation 1272/2008 Of The European Parliament And of Council. classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006, 1(1272), 1056.
- EFSA, E. F. S. A. (2015). Scientific Opinion on Acrylamide in Food -EFSA Panel on Contaminants in the Food Chain (CONTAM). *EFSA J*, 13(16), 4104.
- European commission. (2002). Opinion of the scientific committee on food on new findings regarding the presence of acrylamide in food, European Commission Scientific Committee on Food. Scientific Committee on Food.
- European commission. (2013). Commission Recommendation N^o 647/2013 on investigations into the levels of acrylamide in food. *Official Journal of the European Union*, L301, 17.
- European commission. (2015). Commission Regulation (EU) 2015/705 Laying down methods of sampling and performance criteria for the methods of analysis for the official control of the

levels of erucic acid in foodstuffs and repealing Commission Directive 80/891/EEC.

Official Journal of the European Union, L 113, 29–37.

Evrin, S., Tekkeli, K., & Önal, C. (2012). A Review of Current Methods for the Determination of Acrylamide in Food Products, 29–39. <https://doi.org/10.1007/s12161-011-9277-2>

FAO/WHO. (2010). *Evaluation of certain food contaminants. Seventy-second report of the joint FAO/WHO expert committee on food additives* (Vol. 959). rome. Retrieved from http://www.fao.org/ag/agn/agns/jecfa%7B_%7Dindex%7B_%7Den.asp

Food, W. H. O., & Jecfa, F. A. O. (2011). Safety evaluation of certain contaminants in food- Seventy-second report of the Joint FAO/WHO Expert Committee on Food Additives, 959, 68.

Govaert, Y., Ariseto, A., Van Loco, J., Scheers, E., Fraselle, S., Weverbergh, E., ... Goeyens, L. (2006). Optimisation of a liquid chromatography–tandem mass spectrometric method for the determination of acrylamide in foods. *Analytica Chimica Acta*, 556(2), 275–280. <https://doi.org/10.1016/J.ACA.2005.09.045>

Hilbig, A., Freidank, N., Kersting, M., Wilhelm, M., & Wittsiepe, J. (2004). Estimation of the dietary intake of acrylamide by German infants, children and adolescents as calculated from dietary records and available data on acrylamide levels in food groups, 207.

Hirvonen, T., Jestoi, M., Tapanainen, H., Valsta, L., Virtanen, S. M., Sinkko, H., ... Peltonen, K. (2011). Dietary acrylamide exposure among Finnish adults and children: the potential effect of reduction measures. *Food Additives {&} Contaminants: Part A*, 28(11), 1483–1491. <https://doi.org/10.1080/19440049.2011.593559>

- Hu, Q., Xu, X., Fu, Y., & Li, Y. (2015). Rapid methods for detecting acrylamide in thermally processed foods: A review. *Food Control*, *56*, 135–146.
<https://doi.org/10.1016/J.FOODCONT.2015.03.021>
- IRIS. (2012). *Acrylamide ; CASRN 79-06-1. IRIS Assessment*. Retrieved from
https://cfpub.epa.gov/ncea/iris2/chemicalLanding.cfm?substance_nmbr=286
- Jesus, S., Delgado, I., Rego, A., Brandão, C., Galhano, R., & Castanheira, I. (2018). Determination of Acrylamide in Portuguese Bread by UPLC- MS / MS : Metrological and Chemometric tools, *7*(2), 96–101.
- Kim, C. T., Hwang, E.-S., & Lee, H. J. (2007). An improved LC-MS/MS method for the quantitation of acrylamide in processed foods. *Food Chemistry*, *101*(1), 401–409.
<https://doi.org/10.1016/J.FOODCHEM.2005.10.025>
- Kostoff, R. N., Goumenou, M., & Tsatsakis, A. (2018). The role of toxic stimuli combinations in determining safe exposure limits. *Toxicology Reports*, *5*(November), 1169–1172.
<https://doi.org/10.1016/j.toxrep.2018.10.010>
- Mojska, H., Gielecinska, I., Szponar, L., & Ołtarzewski, M. (2010). Estimation of the dietary acrylamide exposure of the Polish population. *Food and Chemical Toxicology*, *48*(8–9), 2090–2096. <https://doi.org/10.1016/j.fct.2010.05.009>
- Motaghi, M. M., Seyedain Ardebili, M., Honarvar, M., Mehrabani, M., & Baghizadeh, A. D. (2012). Determination of Acrylamide in Selected Types of Iranian Breads by SPME Technique. *Journal of Food Biosciences and Technology Science and Research Branch*, *2*, 57–64.

- Mottram, D. S., Wedzicha, B. L., & Dodson, A. T. (2002). Food chemistry: Acrylamide is formed in the Maillard reaction. *Nature*, *419*(6906), 448–449.
<https://doi.org/10.1038/419448a>
- Mustafa, A. (2008). *Acrylamide in Bread, Precursors, Formation and Reduction*. Swedish University of Agricultural Sciences.
- National Nutrition and Food Technology Research Institute. (2004). *Comprehensive study of food basket pattern, and nutrition status in Iran during 2000-2002*. Tehran: Shahid Beheshti University of Medical Sciences.
- Negoita, M., Culețu, A., Negoita, M., & Culetu, A. (2016). Application of an Accurate and Validated Method for Identification and Quantification of Acrylamide in Bread, Biscuits and Other Bakery Products Using GC-MS/MS System. *Journal of the Brazilian Chemical Society*, *27*(10), 1782–1791. <https://doi.org/10.5935/0103-5053.20160059>
- Norouzi, E., Kamankesh, M., Mohammadi, A., & Attaran, A. (2018). Acrylamide in bread samples: Determining using ultrasonic-assisted extraction and microextraction method followed by gas chromatography-mass spectrometry. *Journal of Cereal Science*, *79*, 1–5.
<https://doi.org/10.1016/J.JCS.2017.09.011>
- Pico, J., Bernal, J., & Gómez, M. (2015). Wheat bread aroma compounds in crumb and crust: A review. *Food Research International*. Elsevier.
<https://doi.org/10.1016/j.foodres.2015.05.051>
- Pugajeva, I., Zumbure, L, A. M.-9th B. C., & 2014, U. (2014). Determination of acrylamide levels in selected foods in Latvia and assessment of the population intake. *Researchgate.net*.

- Pugajeva, I., Zumbure, L., Melngaile, A., & Bartkevics, V. (2014). *FOODBALT 2014 DETERMINATION OF ACRYLAMIDE LEVELS IN SELECTED FOODS IN LATVIA AND ASSESSMENT OF THE POPULATION INTAKE*, 111–116.
- Rivadeneira-domínguez, E., Becerra-contreras, Y., & Vázquez-luna, A. (2018). Alterations of blood chemistry, hepatic and renal function, and blood cytometry in acrylamide-treated rats. *Toxicology Reports*, 5(October), 1124–1128.
<https://doi.org/10.1016/j.toxrep.2018.11.006>
- Sadeghi, E., Yeganeh, S. M., Shoeibi, S., Amirahmadi, M., Karami, F., & Sharafi, K. (2016). Determination of acrylamide in traditional and industrial breads: A case study: Tehran, Iran. Retrieved from <http://eprints.kums.ac.ir/624/>
- SANTE, E. commission. (2017). *Analytical quality control and method validation procedures for pesticide residues analysis in food and feed*. Retrieved from https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_mrl_guidelines_wrkdoc_2017-11813.pdf
- Shahrbabki, P. E., Hajimohammadi, B., Shoeibi, S., Elmi, M., Yousefzadeh, A., Conti, G. O., ... Mousavi Khaneghah, A. (2018). Probabilistic non-carcinogenic and carcinogenic risk assessments (Monte Carlo simulation method) of the measured acrylamide content in Tahdig using QuEChERS extraction and UHPLC-MS/MS. *Food and Chemical Toxicology*, 118(April), 361–370. <https://doi.org/10.1016/j.fct.2018.05.038>
- Sirot, V., Hommet, F., Tard, A., & Leblanc, J.-C. (2012). Dietary acrylamide exposure of the French population: Results of the second French Total Diet Study. *Food and Chemical*

- Toxicology*, 50(3–4), 889–894. <https://doi.org/10.1016/j.fct.2011.12.033>
- Stadler, R. H., Blank, I., Varga, N., Robert, F., Hau, J., Guy, P. A., ... Riediker, S. (2002). Acrylamide from Maillard reaction products. *Nature*, 419(6906), 449–450. <https://doi.org/10.1038/419449a>
- Statista. (2013). Volume of bread consumed per person per year in selected European countries in 2013 (in kilograms).
- Storelli, M. M. (2008). Potential human health risks from metals (Hg, Cd, and Pb) and polychlorinated biphenyls (PCBs) via seafood consumption: Estimation of target hazard quotients (THQs) and toxic equivalents (TEQs). *Food and Chemical Toxicology*, 46(8), 2782–2788. <https://doi.org/10.1016/j.fct.2008.05.011>
- Tareke, E., Rydberg, P., Karlsson, P., Eriksson, S., & Törnqvist, M. (2002). Analysis of acrylamide, a carcinogen formed in heated foodstuffs. *Journal of Agricultural and Food Chemistry*, 50(17), 4998–5006. <https://doi.org/10.1021/jf020302f>
- Taylor, P., Rufian-henares, J. A., Arribas-lorenzo, G., & Morales, F. J. (2007). Acrylamide content of selected Spanish foods : Survey of biscuits and bread derivatives, (May 2013), 37–41. <https://doi.org/10.1080/02652030601101169>
- Tsatsakis, A. M., Kouretas, D., Tzatzarakis, M. N., Stivaktakis, P., Tsarouhas, K., Golokhvast, K. S., ... Tsitsimpikou, C. (2017). Simulating real-life exposures to uncover possible risks to human health: A proposed consensus for a novel methodological approach. *Human and Experimental Toxicology*, 36(6), 554–564. <https://doi.org/10.1177/0960327116681652>

- Virk-Baker, M. K., Nagy, T. R., Barnes, S., & Groopman, J. (2014). Dietary Acrylamide and Human Cancer: A Systematic Review of Literature. *Nutrition and Cancer*, 66(5), 774–790. <https://doi.org/10.1080/01635581.2014.916323>
- Zhuang, H., Zhang, T., Liu, J., & Yuan, Y. (2012). Detection of acrylamide content in traditional Chinese food by high-performance liquid chromatography tandem mass spectrometry method, 6337. <https://doi.org/10.1080/19476337.2010.538861>

Table 1. Performance characteristic data of the optimized method (n = 9).

Spike level (ng/g)	Average of Recovery (%)	CV (%)	Uncertainty (%)
1.50	101	9.85	19.8
30	90.42	6.0	12
50	96.0	10.3	20.6

Table 2. Contamination of Iranian bread samples with acrylamide (ng/g)¹

Location	Bread Type	Acrylamide levels (ng/g)				
		Range	Mean	Median	90 th percentile	97.5 th percentile
Shiraz	Semi-industrial Sangak	25.4-83.3	48.5±13.3	53.2	64.0	76.3
	Traditional Sangak	LOQ-76.4	43.4±22.0	43.8	73.4	75.6
Tehran	Traditional Sangak	5.8-60.6	21.5±14.3	20.3	44.6	54.2
	Industrial	LOQ-19.2	8.0±5.5	7.2	15.2	18.6
Tehran and Shiraz	All samples	LOQ-83.3	24.4	17.9	56.3	71.6

¹ Mean, Mean, 90th percentile and 97.5th percentile of all samples; Data below LOQ (1 ng/g) were assumed to be 0.5 ng/g.

Table 3. Dietary exposure to acrylamide (ng/kg bw/day) through bread consumption in population of Shiraz and Tehran¹.

Location	Bread type	Mean	90 th percentile	97.5 th percentile	Max
Shiraz	Semi-industrial Sangak	200±66.8	252	313	340
	Traditional Sangak	180±90	294	308	312
Tehran	Traditional Sangak	88.0±59	182	221	248
	Industrial	33.0±22.5	61.9	76.1	78.2
Tehran and Shiraz	All samples	127±20	48.9	50	340

¹ Mean, 90th percentile, 97.5th percentile and max of all samples: Data below LOQ (1 ng/g) have been assumed to be 0.5 ng/g.

Note: The mean bread consumption for the urban population, based on the national nutrition survey (National Nutrition and Food Technology Research Institute, 2004) data, was estimated to be 286 g per person per day. In this study, the average concentration of acrylamide was calculated in all samples and LOQ/2 was assumed for the concentrations under LOQ (Food & Jecfa, 2011)

Table 4a. Chronic daily intake (CDI, mg/kg/day) and Target hazard quotient (THQ)¹ of acrylamide for Iranian adults and children posed by the selected bread consumption

Location		Shiraz		Tehran		Tehran and Shiraz
Bread type		Semi-industrial Sangak	Traditional Sangak	Traditional Sangak	Industrial	Mean
Adults	CDI	0.0002219	0.000198	0.0000983	0.0000367	0.000112
	THQ	0.1109257	0.099131	0.049188571	0.018331	0.055771
Children	CDI	0.001035	0.000925	0.000459	0.000171	0.000521
	THQ	0.517653	0.462613	0.229547	0.085547	0.260267

¹ THQ lower than 1 indicates no risk for human health derived from consumption (Adel et al., 2016).

Table 4b. Incremental Lifetime Cancer Risk (ILCR) of acrylamide for Iranian adults and children posed by the selected bread consumption

Location	Shiraz		Tehran	
	Semi-industrial Sangak	Traditional Sangak	Traditional Sangak	Industrial
Adults ILCR	11.1E-5	9.9E-5	4.91E-5	1.83E-5
Children ILCR	51.76E-5	46.26E-5	22.95E-5	8.55E-5

Table 5. Occurrence of acrylamide in bread: Results of a number of international Surveys

Matrix	Publication date	Analytical method	Concentration ($\mu\text{g}/\text{kg}$)	Ref
Bread (Syria)	2016	UPLC-MS	119 \pm 1.73 - 263 \pm 3.6	(Alyousef, Wang, Al-Hajj, & Koko, 2016)
Whole wheat bread (Romania)	2016	GC-MS/MS	56.9 \pm 0.3	(Negoita, Culețu, Negoita, & Culetu, 2016)
Wheat bread samples (Turkey)	2015	LC-MS/MS	28.97	(Alpozen et al., 2015)
Wheat bread (Latvia)	2014	UPLC-MS/MS	14	(I Pugajeva et al., 2014)
Bread (Korea)	2007	LC-MS/MS	33.0 \pm 4.9	(Kim et al., 2007)
Bread (Germany)	2004	-	916	(Hilbig et al., 2004)
Oil-based sweet bread (Spain)	2008	LC-MS/MS	113	(Bermudo, Moyano, Puignou, & Galceran, 2008)
Crispbread (Spain)	2007	LC-MS	About 100	(Taylor et al., 2007)
Bread (Belgium)	2006	LC-MS/MS	118	(Govaert et al., 2006)
Crisp bread (Sweden)	2008	-	About 300	(Mustafa, 2008)
Whole grain bread (Portugal)	2018	LC-MS/MS	787	(Jesus et al., 2018)
Bread (China)	2012	LC-MS/MS	About 35	(Zhuang et al., 2012)

Table 6. Occurrence of Acrylamide in different types of bread in Iran

No.	Publication date	Analytical Instrument	Measured value ($\mu\text{g}/\text{kg}$)	Ref.
1	2018	GC-MS	165.62 ± 8.94 to 289.72 ± 15.64	(Norouzi, et al., 2018)
2	2016	LC-MS/MS	Sangak (139.3) Hamburger buns (40.9)	(Sadeghi et al., 2016)
3	2015	GC-MS	Bread roll (239.1 ± 1.9) Sangak bread (135.06 ± 3.3)	(Dastmalchi et al., 2016)
4	2012	GC-FID	Taftoon(146), Sangak(86), lavash (26) and Barbari (135)	(Motaghi et al., 2012)

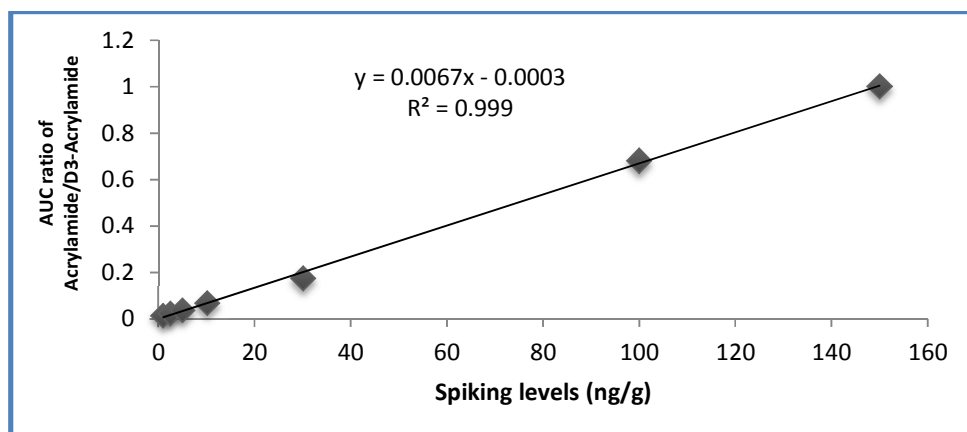


Figure 1. Calibration curve of acrylamide using bread samples spiked (with acrylamide)

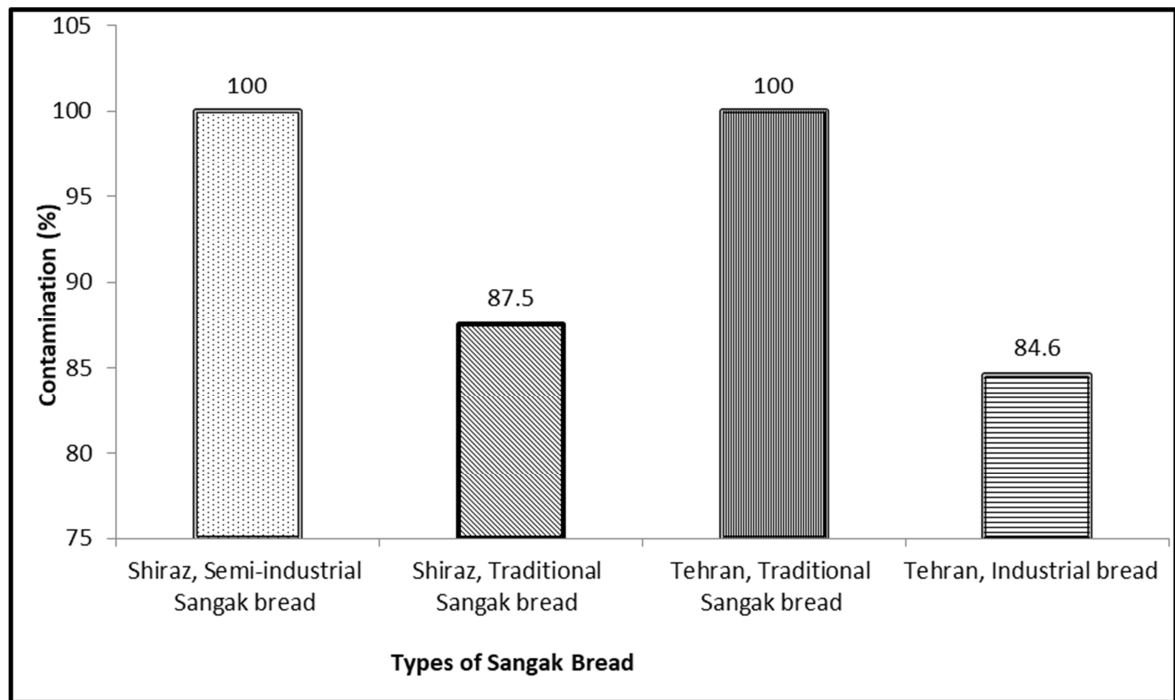


Figure 2: Incidence of acrylamide in different Sangak bread samples collected from Tehran and Shiraz, IR Iran.

Highlights

- Monitoring of acrylamide in different bread types using effective method is necessary.
- Bread is a major source for acrylamide intake by people in Tehran and Shiraz.
- The Incremental Lifetime Cancer Risk of acrylamide for adults and children consuming wheat flour bread was higher than the permissible lifetime carcinogenic risk.
- All consumers regardless of age are at elevated carcinogenic risk.