



## ASSESSMENT OF FOOD ADDITIVES IMPACT ON ACRYLAMIDE FORMATION IN POPCORN SUPPLIED IN TEHRAN, IRAN: A RISK ASSESSMENT STUDY

Razieh Shahbazi<sup>1</sup>, Behrouz Akbari Adergani<sup>2</sup>, Nabi Shariatifar<sup>1</sup>, Khadijeh Jafari<sup>3</sup>, Ensiyeh Taheri<sup>3</sup>, Ayub Ebadi Fathabad<sup>4</sup>, Naiema Vakili Saatloo<sup>5</sup>, Ebrahim Molae Aghae<sup>1</sup>, Parisa Sadighara<sup>1</sup>✉, Amin Mousavi Khaneghah<sup>6,7</sup>✉

<sup>1</sup>Department of Environmental Health, Food Safety Division, Faculty of Public Health, Tehran University of Medical Sciences, Tehran, Iran.

<sup>2</sup>Food and Drug Laboratory Research Center, Food and Drug Administration, Ministry of Health and Medical Education, Tehran, Iran.

<sup>3</sup>Environment Research Center, Research Institute for Primordial Prevention of Non-communicable Disease, Isfahan University of Medical Sciences, Isfahan, Iran.

<sup>4</sup>Social Determinants of Health Research Center, Department of public health, School of health, Birjand University of Medical Sciences, Birjand, Iran

<sup>5</sup>Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran

<sup>6</sup>Department of Fruit and Vegetable Product Technology, Prof. Wacław Dąbrowski Institute of Agricultural and Food Biotechnology – State Research Institute, 36 Rakowiecka St., 02-532, Warsaw, Poland

<sup>7</sup>Department of Technology of Chemistry, Azerbaijan State Oil and Industry University, Baku, Azerbaijan  
✉ sadighara@farabi.tums.ac.ir

✉ amin.mousavi@ibprs.pl; mousavi.amin@gmail.com

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### ABSTRACT

Acrylamide (AA) is a probable toxic substance that forms naturally, especially in carbohydrate-rich foods. Adding flavorings to foods and food additives makes people new food habits and customer friendly. This study investigated the AA concentration in 34 commercial popcorn samples with different additives. Also, health risk assessment was carried out using the Simulation of Monte Carlo methods. The highest and lowest mean AA concentration was detected in salty popcorn and cheese popcorn, respectively. The differences in acrylamide amounts in popcorn samples are due to differences in the food additives used for each popcorn type production. Hazard quotient (HQ) was determined for three age groups (<18, 18-60, and >60 years old). For all three age groups, mean HQ was lower than 1, but the probabilistic approach has shown that the HQ 90% was higher than 1 for both age groups <18 and 18-60 years old and revealed that AA might have potential health problems. Cancer risk for all three age groups indicates a potential concern for consumers. Therefore, controlling and monitoring AA demand in the different flavors and brands is essential, especially for the children group.

## 1. Introduction

The contamination of food products with pollutants such as heavy metals and mycotoxins raised notable concerns during last decade

(Bangar, Sharma, Bhardwaj, & Phimolsiripol, 2022; Bounar, Boukaka, & Leghouchi, 2020; Gao et al., 2022; Heshmati, Mehri, Karami-Momtaf, & Khaneghah, 2020; L. Hu et al.,

2022; Khaneghah et al., 2023; Luo et al., 2022; Pires et al., 2022; Rezaei et al., 2020). However, the issue of contaminants in food products also is associated with other toxic compounds like acrylamide (Atabati et al., 2020; Hwang & Kwon, 2022; Mousavi Khaneghah, Fakhri, Nematollahi, Seilani, & Vasseghian, 2020; Nematollahi et al., 2020a; Nematollahi, Meybodi, & Khaneghah, 2021a; P. E. Shahrabaki et al., 2018; Zokaei et al., 2020). One of the neurotoxin compounds is Acrylamide (AA) (Costa, Freita, Mendes, Roviero, & Mutton, 2018; Hwang & Kwon, 2022). This compound is classified as a "probable human carcinogen" and as well as listed with IARC (international agency for cancer research) as a carcinogenic compound through experimental studies (Bušová, Bencko, Kromerová, Nadjo, & Babjaková, 2020; Deribew & Woldegiorgis, 2021; Hamid, Yaqub, Ahmed, & Aziz, 2017; Mucci & Wilson, 2008). Since the 1950s, it has been utilized in the chemical industry to manufacture polyacrylamide polymers and enhance polymers' adhesive bonding. Notably, incomplete polymerization processes may result in the residual monomer of AA in products; also, the carcinogenic effect happened at the maximum concentration limit of 5 mg/kg (Khan, Alothman, Naushad, Alomary, & Alfadul, 2018; Medeiros Vinci, Mestdagh, & De Meulenaer, 2012). Rather than being a food contaminant, the primary way to create AA in foods is through a sequence of Maillard reactions involving an amino acid, mainly asparagine, and a reducing sugar such as glucose or fructose during cooking. Such conditions, including temperature, can affect the formation of AA, which can occur in some foodstuffs during high-temperature cooking operations at temperatures between 120°C and 180° C (Mottram, Wedzicha, & Dodson, 2002; Nematollahi, Meybodi, & Khaneghah, 2021b). As a result, AA is formed during thermal processing such as (Mohsen et al., 2020)roasting, frying, and baking. Thus, the European Union (EU), in 2007-2009, suggested that member states conduct annual monitoring of AA levels in specific foods including drinking water, potato crisps, fresh potato and dough, popcorn, type of

coffee for control and monitoring the permissible limit of AA according to µg/kg (Authority, 2012; Koszucka, Nowak, Nowak, & Motyl, 2020; Ma et al., 2016; Pacetti et al., 2015; Park, 2021; Wenzl & Anklaam, 2007). For example, according to the mean body weight of people in several counties such as Italy, Germany, and the Netherlands, the recommended average daily intake of AA was 0.45, 0.32, and 0.32 µg/kg bw per day, respectively (Koszucka et al., 2020).

The AA presence in a range of heat-treated foods is relatively low, and the risk of carcinogenicity is considerable (Peivasteh-Roudsari et al., 2022). Increasing snacking and fast-food consumption lead to a high AA intake. In addition, in several studies, the presence of AA in popcorn and corn seeds was measured (Baskar & Aiswarya, 2018; Bocharova, Reshta, & Bocharova, 2017; Khan et al., 2018). Since the different types of popcorn with different flavours are among the most consumed foodstuffs among adults and especially children in Iranian people (Shaviklo, Dehkordi, & Zangeneh, 2015). Making popcorn invariably entails processing at high temperatures, which builds enough pressure within the kernels to cause them to burst (Bocharova et al., 2017; Byrd & Perona, 2005).

Although remarkable contention exists as to exposure levels associated with AA's carcinogenicity in humans, data on the presence of AA in foods needs to be analysed. For the importance of AA monitoring amounts in the different types of foods, new technologies have developed and increased AA exposure through the intake of roasted foods, seeds, and popcorns in recent years. In recent years, numerous published methods for determining the quantity of AA monomer have been developed, including distinct extraction methods in different food matrixes. Firstly, the presence of AA was determined in a variety of heat-treated meals using the isotope dilution liquid chromatography-tandem mass spectrometry (LC-MS/MS) approach (Byrd & Perona, 2005; F. Hu et al., 2017; Kamankesh, Nematollahi, Mohammadi, & Ferdowsi, 2021; Sun, Fang, & Xia, 2012). Also, other techniques such as

HPLC, CE-MS (Capillary Electrophoresis-Mass Spectrometry), NACE (Non-Aqueous Capillary), HPLC-MS (High-Performance Liquid Chromatography-Mass Spectrometry), GC-MS (Gas Chromatography-Mass Spectrometry), Solid-Phase Micro Extraction-Gas Chromatography (SPME-GC), MSPD (Matrix Solid-Phase Dispersion), ELISA methods (Enzyme-Linked Immunosorbent Assay), and Micro Emulsion Electro Kinetic Chromatography (MEEKC) were as standard methods to AA and other contaminant detection (Arvanitoyannis & Dionisopoulou, 2014; De Souza, Khaneghah, & Oliveira, 2021; Maqbool et al., 2021; Tang, Qu, Cao, Wang, & Lou, 2020). However, many food samples must be examined and analysed to determine AA. Although in this case, it is vital to develop a reliable and low-cost analytical approach for determining AA (Mollakhalili-Meybodi, Khorshidian, Nematollahi, & Arab, 2021).

For the first time, AA concentrations in popcorn, a product consumed widely by children and adults in Iran, were found in this study.

## 2.2. Instruments

A Hewlett Packard (HP) 1090 M type HPLC system (UV-vis) with a quaternary pump and vacuum degasser was used to quantify AA. Chromatographic separations were carried out on a 25cm×4, 6 Mm Zorb ax ODS (5m) column. The blank sample was analyzed for confirmation using the same device linked to a UV diode array detector (UV-DAD). The flow rate of the mobile phase (acidic water) was adjusted to 0.5 mL/min during routine analysis of food samples. At 202 nm, the absorbance of AA was determined.

Acrylamide spiked Chromatogram in popcorn (with HPLC-UVvis chromatogram), the analyses were performed by a sensitive HPLC equipped with a UV-vis detection system. There were several clean-ups and removing interference steps in the used procedure and an enrichment phase that we dried the extracted sample and reconstituted it with an appropriate solvent that was finally injected into the HPLC. Some other reports revealed obtaining adequate LOQ to quantirandomly from several places in Tehran with different

Electrophoresis), LC-MS, Pressurized Fluid Extraction (PFE

Thus, the primary objective of this study is to identify the probability of AA production in popcorn with different food additives in the different brands, with HPLC (UV-DAD), and to estimate the health risk of carcinogenic and non-carcinogenic disease was also determined by Monte Carlo Simulation (MCS) (Heshmati, Khorshidi, & Khaneghah, 2021; Heshmati et al., 2020; Jafari et al., 2021; Makkaew, Chaloeijitkul, & Vattanasit, 2022; Tang et al., 2020).

## 2. Materials and Methods

### 2.1. Chemicals and solutions

Sigma provided Acrylamide (>99 percent) (Deisenhofer, Germany). Methanol, acetonitrile, acetic acid, and acetone were of analytical grade, while Merck supplied potassium hexacyanoferrate (Carrez I) and zinc sulfate (Carrez II) (Darmstadt, Germany). The AA content was determined using distilled, double deionized, and 0.20 µm filtered water.

production dates to extract AA. fy such low AA levels in foodstuffs by HPLC-UV detection (H. Wang, Feng, Guo, Shuang, & Choi, 2013).

### 2.3. Sampling

Five popular Iranian popcorn products with four flavours were collected (6-8 each) and alphabetically nominated (A, B, C, D, and E). All popcorn samples were collected.

### 2.4. Sample Preparation

The AA content was achieved using Shahrabaki Peah, B. et al. (2018) and Oroian, M.S., (2015) methods with some modifications (Oroian, Amariei, & Gutt, 2015a; P. E. a. H. Shahrabaki, B. and Shoeibi, S. and Elmi, M. and Yousefzadeh, A. and Conti, G.O. and Ferrante, M. and Amirahmadi, M. and Fakhri, Y. and Mousavi Khaneghah, A. (2018) Probabilistic non-carcinogenic and carcinogenic risk assessments (Monte Carlo simulation method) of the measured acrylamide content in Tah-dig using QuEChERS extraction and UHPLC-MS/MS. *Food and Chemical Toxicology*, 118. pp. 361-370). At first, samples were smashed (less than 1 mm), and 1 gram of each sample was put into a 50 mL centrifuge tube. Samples were

mixed well with 100  $\mu\text{L}$  acetamide as internal standard (Kim, Hwang, & Lee, 2011), and mixed with appropriate AA solutions at different concentrations to assess the percentage of relative recovery. Each sample was shaken enough and aqueous phase-separated in another falcon tube from solid samples. It is critical to eliminate solid remnants from the supernatant thoroughly, and samples were centrifuged again if necessary. Then 5 mL deionized water, 5 mL acetonitrile, and 2.5 mL hexane were applied to remove protein and a fat portion from samples. The sample tube was shaken for 30 minutes using a shaker.

Then samples were centrifuged at 3000 rpm (30 min) at 250 rpm. Centrifugation separates the sample tubes into three layers: the top layer (which includes proteins), the middle layer (which contains acrylamide), and the bottom layer (which contains acrylamide) (sample precipitate). The upper-fat layer was removed, and middle layer containing AA was transferred into a clean test tube. This will separate the water-soluble co-extractive components from the pure aqueous phase.

The separated phase was treated with 1 mL Carrez I and II solutions to precipitate the remained protein and soluble carbohydrates (Norouzi, Kamankesh, Mohammadi, & Attaran, 2018). After centrifuging the sample for 30 minutes at 250 rpm, the supernatant was transferred to a conical flask and evaporated until dry in an evaporator. The residue was dissolved in (acidic water concentration of 4 mL with  $\text{pH}=3$ ) for 2 minutes using a vortex. Thus, following shaking (250 rpm) and centrifuging for 20 minutes at 3000 rpm, the AA residue was wholly dissolved in the water phase, while the leftover lipids and lipid-soluble co-extractive chemicals were separated into the test tube wall. Filtration of the fluid was performed using a 0.2  $\mu\text{m}$  syringe filter. Twenty microliters of the finished solution were loaded into a high-performance liquid chromatography column for quantification (Ghalebi, Hamidi, & Nemati, 2019; Sarion, Dabija, & Codină, 2020).

## 2.5. Statistical analysis

SPSS software version 22 was used to

conduct statistical analyses. The independent T-test and one-way analysis of variance (ANOVA) were used to compare the mean concentration of AA in different brands with different flavours. Additionally, to determine the probabilistic health risk, MCS was performed using Crystal Ball software (version 11.1.1.1, Oracle, Inc, USA) with a total of 10,000 trials.

## 2.6. Monte Carlo simulation for Health risk assessment

The current study assessed the risk of carcinogenic and non-carcinogenic effects of acrylamide in various brands and flavours of popcorn (Eisenbrand, 2020; Sanaei et al., 2021) (Eq 1-3).

$$EDI = \frac{C \times IR}{BW} \quad (1)$$

Where EDI represents the estimated daily intake of AA (mg/kg body weight. Day), C represents the concentration of AA in investigated samples (mg.  $\text{kg}^{-1}$ ), IR represents the popcorn intake rate for each age group (kg/day), and BW represents the reference body weight (kg) (Morales-Moo et al., 2020).

Hazard quotients (HQ) were determined by Eq (2) to assay the potential non-carcinogenic health risk of popcorn consumption containing AA.

$$HQ = \frac{EDI}{RfD} \quad (2)$$

HQ is the target hazard quotients (Almendares Calderon, Garcia Mena, & Roman Miranda, 2020; Hamid, Wasim, Azfar, Amjad, & Nazir, 2020; Qasemi et al., 2022; Yao, Lin, Yan, Huang, & Chen, 2021), whereas RfD denotes the reference dosage of acrylamide (2 ng/kg/day) (Nematollahi et al., 2020b). When the  $HQ > 1$  indicates a potential risk for consumers, and when the  $HQ < 1$ , popcorn consumption is the safe level for non-carcinogenic risk. This study evaluated lifetime carcinogenic risk (LTCR) using Eq (3).

$$LTCR = EDI \times CSF \quad (3)$$

CSF is the cancer slop factor for acrylamide that is 0.5 mg/kg of the day (Eslamizad et al., 2019; Nematollahi et al., 2020b). The

parameters used to determine the health risk of acrylamide in industrial popcorn are shown in Table 1.

**Table 1.** Distributions of parameters for different age groups used in risk assessment model with Monte Carlo simulation.

Parameters	Unit	Description	Distribution	age groups (year)			Ref
				<18	>18 <60	>60	
EDI	mg/kg/day	daily intake through ingestion	-	Will be assessment with Eq. (1)			(Morales-Moo et al., 2020)
HQ	-	Target Hazard Quotient Index	-	Will be assessment with Eq. (2)			(Nematollahi et al., 2020)
C	mg/kg	Acrylamide Concentration	Log normal	3.45 ± 3.52			-
IR	g/day	ingestion rate	Log normal	28 ± 5	23 ± 41	5 ± 3	(Morales-Moo et al., 2020)
BW	Kilogram	Mean of Body weight	Log normal	52 ± 13	66 ± 12	67 ± 10	(Morales-Moo et al., 2020)
RfD	µg/kg/day	Oral reference dose	Fixed value	2			(Nematollahi et al., 2020)

### 3. Results and discussion

#### 3.1. The concentration of Acrylamide in the different brands with different flavours

The concentration of acrylamide in several brands with varying tastes. The presence of acrylamide in a variety of popular Iranian popcorn products was determined in this investigation. Microwave popcorn manufactured in Iranian facilities consists of a microwave popping bag, kernel popcorn, fat, and, in most cases, salt, vinegar, cheese, ketchup. Popcorn was made by combining

kernel popcorn, salt, and melted fat into a paste and dropping the paste into the selected chamber of the popcorn bag prior to the final closing. Recently, microwave popcorn products have grown in popularity, and as a result, their acrylamide concentration must be determined.

The results of identifying acrylamide concentrations in popcorn products are presented in Table 2. AA level in popcorn contains food additives is variable between 0.23±0.32 mg/kg to 8.9±1.9 mg/kg. There is no difference between Brand A, B, C, D, and E (p>0.05).

**Table 2.** Acrylamide concentrations in industrial popcorns with various flavorings agent in different brands (mg/kg).

Flavoring's agent	Brand A	Brand B	Brand C	Brand D	Brand E
Salty popcorn	1.8±1.4	1.5±0.04	-	4.7±6.4	8.9±1.9
Vinegar popcorn	-	1.8±0.13	0.96±0.62	-	5.6±7.9
Cheese popcorn	2.5±0.2	1.4±0.09	0.86±0.01	0.23±0.32	4.8±5.7
Ketchup popcorn	2.2±1.6	1.4±0.24	1.04±0.36	5.5±6.7	8.1±1.8

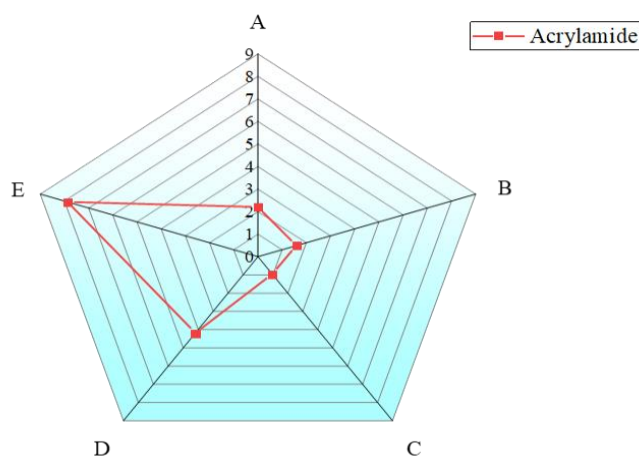
The formation of AA was dependent on several parameters, including raw ingredients, extrusion circumstances, and the presence of CO<sub>2</sub> (Masatcioglu, Gokmen, Ng, & Koksels,

2014). The monosaccharides found in maize endosperms, such as glucose, fructose, galactose, ribose, and mannose, are the Maillard reactions' initial stage (Inglett, and, & Press.).

The addition, food additives affected AA levels in packaged popcorn. Some additives are considered safe or beneficial to consumers, especially popcorn with low AA levels. For example, in the recent study in Iran, AA concentration was evaluated in the snack samples, that AA levels were generated faster in potato-based samples than in corn-based samples. Thus, compared to the other two variables, the composition of potato-based samples had the most significant influence (Kamankesh et al., 2021). Numerous research has been conducted on the amount of AA found in various food products worldwide. Abramson et al. found that AA level in popcorn was 500  $\mu\text{g}/\text{kg}$  (Svensson et al., 2003), and Das et al. detected 400  $\mu\text{g}/\text{kg}$  AA levels in popcorn that meet data in this survey (Das & Srivastav, 2012). In the study by Sanchez-oter et al., in 2017, the highest amount of AA was 5.80 mg/kg for potato chips, so the AA concentration differs according to the type of food and cooking condition (Sanchez-oter et al., 2017). In other words, the level of sugars in the different foods, especially in potato, cereal, and coffee products, depended on various factors and parameters, including the type of food, compounds of these food, heating and cooking process, and condition of storage of this food. Also, adding some additives during cooking reduces AA

formation (Pedreschi, Mariotti, & Granby, 2014).

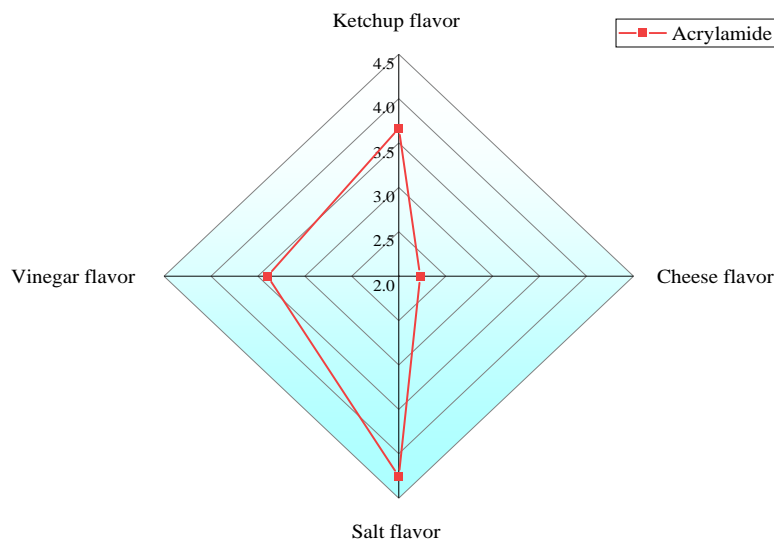
Among 5 additives into popcorn samples, the mean concentration of AA according to different brands of popcorn shows in Figure 1, that highest concentration of AA was in the E > D > A > B > C. As indicated by the data, the quantity of AA in popcorn products is higher in Brand E (8.91.9 for salty tastes has the greatest concentration) than in others, which may be related to differences in manufacturing methods. Popcorn in Brand E is heated to 200–220 °C for 2 minutes, whereas samples A, B, C, and D are heated to 180–200 °C for less than 2 minutes. Compared to the other samples, Sample C with vinegar flavour contains low AA. However, extreme caution must be exercised when picking food additives with kernel popcorn. The influence of various food additives used in popcorn production on the development of AA is still being investigated. In the case of recipes, considerable cost savings can be gained by substituting the chemical raising agent ammonium bicarbonate for the comparable sodium salt in items such as gingerbread (Lineback, Coughlin, & Stadler, 2011). However, since the focus on AA was globally pointed out about health risks (around the year 2002), there has been a gradual reduction of AA in snacks (Al-Jawaldeh et al., 2021; Başaran, 2020; Soares, 2015).



**Figure 1.** AA mean concentration in the popcorn according to type of brand

Because AA in food raises cancer risk for consumers of all ages, mitigating techniques are used to keep AA levels in food as low as possible. Mitigating AA in popcorn products without lowering dietary AA intake (Bušová et

al., 2020; Maan et al., 2020). Mean concentration of AA according to different flavours of popcorn shows in Figure 2 that the highest concentration of AA was in the salty> ketchup> vinegar> cheese flavours.



**Figure 2.** Mean concentration of AA in the popcorn according to different flavours.

A study by Eslamizad et al. (Eslamizad et al., 2019), shows the mean concentration of AA in the different bread with the different conditions of cooking. Their average AA concentration in the semi-industry trial sangak bread and traditional sangak in Shiraz were 48.5 and 43.4 ng/g, respectively. Also, the amount of AA according to the location and eating habits of people from different countries are different as well as age, sex, income, and exposure to passive smoking (Alpözen, Güven, Özdehan, & Üren, 2015; Alyousef, Wang, Al-Hajj, & Koko, 2016; Choi, Ko, Kang, Hwang, & Lee, 2019; Khan et al., 2018; Norouzi et al., 2018; Sadeghi et al., 2016).

Comparing this study, the type of food and amount of surges compound are the factors that affected the AA concentration rather than our study. Another study measured AA in different food. Their results show that the AA mean concentration in the corn-based extruded snack was about 257 ng/g, which was significantly lower than potato crisps (1162 ng/g) and significantly higher than Roasted peanuts (21 ng/g). Also, AA concentration in the corn-based

extruded snack was lower than AA levels in the salty flavour of popcorn in brand E (Esposito, Nardone, Fasano, Triassi, & Cirillo, 2017). The method of popcorn cooking is a critical component in reducing the production of AA. Thus, the existence of D-allose in microwaved popcorn was established. AA has not discovered infractions of popcorn treated for 2, 5, or 8 minutes in a microwave oven. The absence of asparagine, a critical component of AA synthesis, in the pericarp of maize kernels was the primary factor affecting the results obtained (Bocharova et al., 2017). AA concentration was measured in several foods such as corn breakfast cereal (498 µg/kg) and microwave popcorn (607 µg/kg) in the México

## 3.2. Health risk assessment

### 3.2.1. Non-carcinogenic risk

The rank order of EDI (mg/kg/day) for age groups were lower than 18 years ( $1.86E-3$ )> age group 18-60 years ( $1.2E-3$ ) > age group higher than 60 years old ( $2.57E-4$ ), respectively.

According to the results shown in Figure 3, for all of the samples in the all-age groups (age

<18, age >18<60, and age > 60 years old), the mean value of HQ was lower than 1, which indicate there was no concern about non-carcinogenic risk due to acrylamide consumption through industrial popcorn with different flavors and brands.

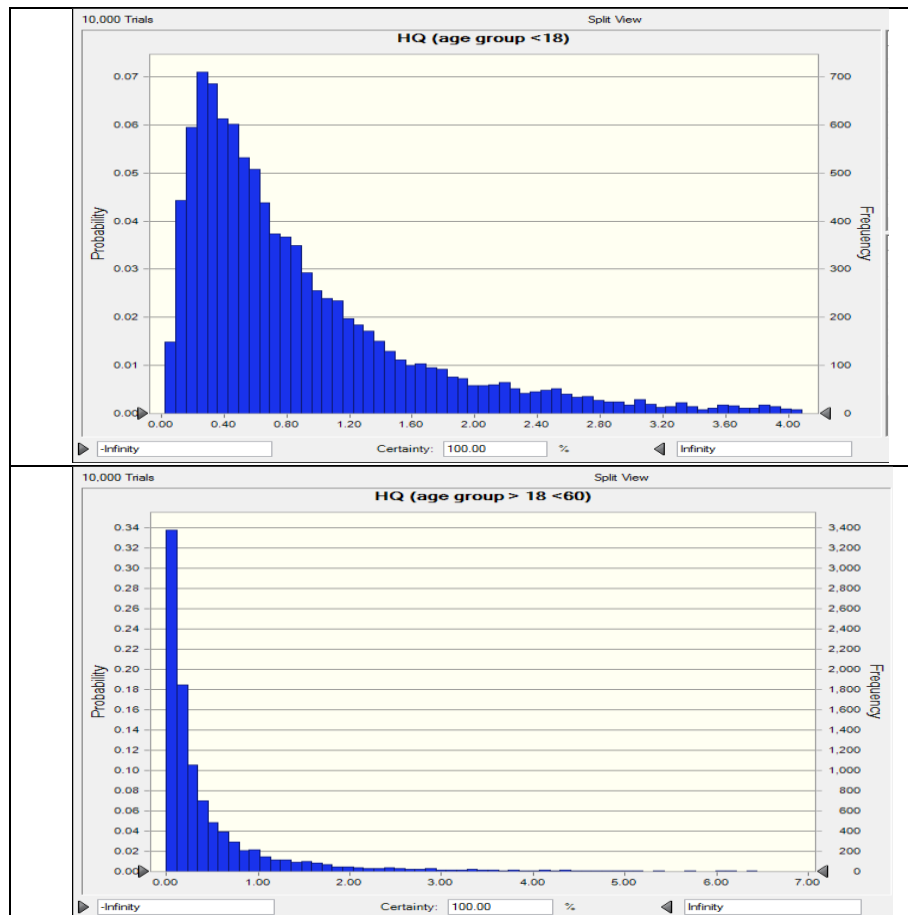
Factors such as the condition of cooking, including traditional and industrial processes, are the foremost important parameters in the formation of AA. Also, the time of heating and temperature of cooking and type of food is vital to reduce the HQ due to AA intake (Eslamizad et al., 2019); similar to these studies for all age groups, the mean value of HQ was less than 1. It is notable that in this study, while the mean

value of HQ was lower than 1, the results of MCS in Table 3 indicated that the percentile 90% HQ values for age groups <18 and 18- 60 years old was higher than 1, which indicated potential health risk for these population (Naeem et al., 2022; SHARIF & NAZIR, 2018).

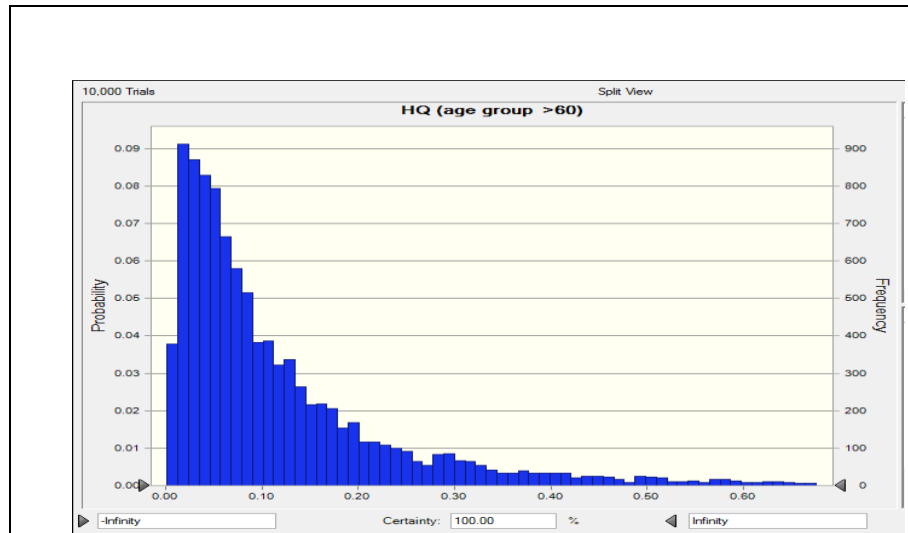
The only noncarcinogenic and non-genotoxic effect of AA in humans is neurotoxicity (oral intake from food). However, in animal experiments, AA causes testicular injury and detrimental effects on fertility. In vivo, AA is genotoxic to somatic and germ cells and can cause heritable gene and chromosome damage (Park, 2021; B. Wang, Guerrette, Whittaker, & Ator, 2020; WHO, 2002)

**Table 3.** Hazard quotient values of AA exposure levels for different age groups.

	Age group	50%	90%	Mean
Probabilistic approach	< 18	0.65	2.10	0.98
	> 18 < 60	0.21	1.43	0.66
	> 60	0.08	0.30	0.13



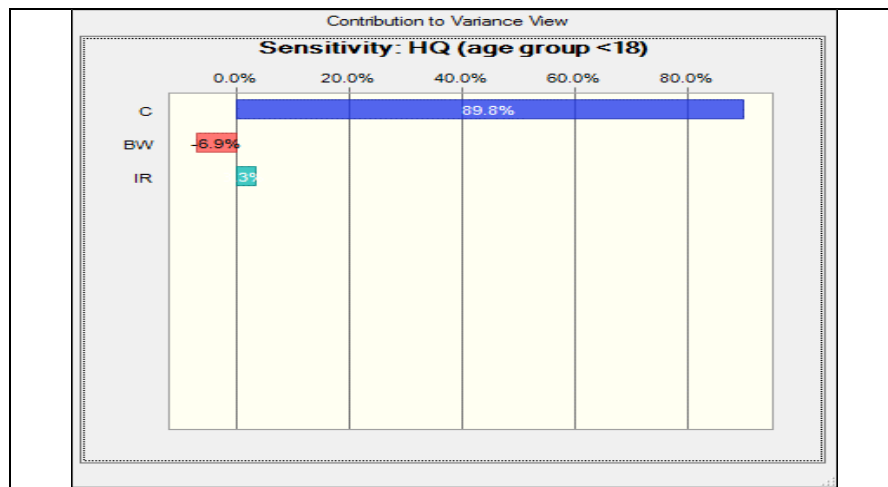


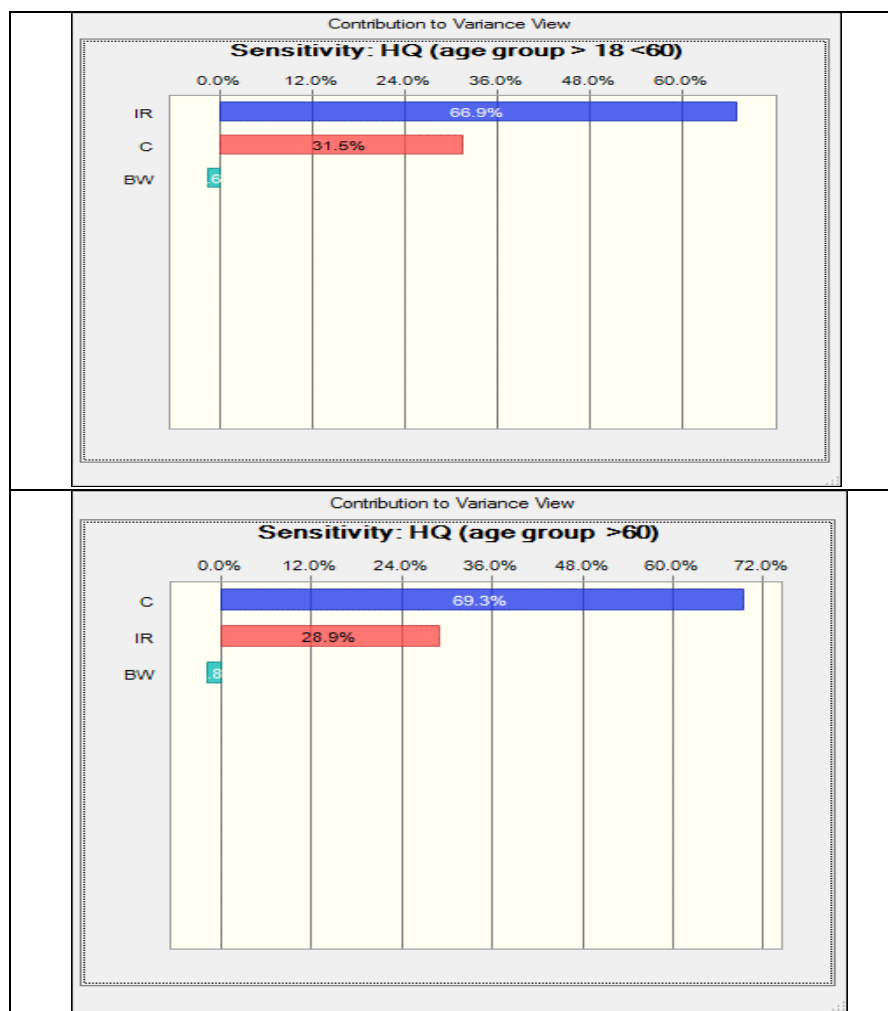


**Figure 3.** Hazard Quotient Index (HQ) of Acrylamide for three age groups (<18, > 18 <60, and >60 year) (The result of Crystal Ball, HQ defined as a Forecast and the assumptions were IR, BW, and C, calculated by 10000 trials number).

Figure 4 depicts a sensitivity analysis of noncarcinogenic risk for three age groups. Sensitivity analysis was used to discover the most influential risk factors for noncarcinogenic illnesses. The results for the age groups tested are as follows: among those aged 18 to 60 years,

the most influential parameter is AA concentration in the examined samples. The volume of popcorn consumed was the most influential factor in the age range > 18 - 60 years old. However, the content of acrylamide played a significant influence as well.





**Figure 4.** Sensitivity analysis of noncarcinogenic health risk for three age groups (<18, > 18 <60, and >60 year) (The result of Crystal Ball software with 10000 trails, HQ defined as a Forecast and the assumptions were IR, BW and C).

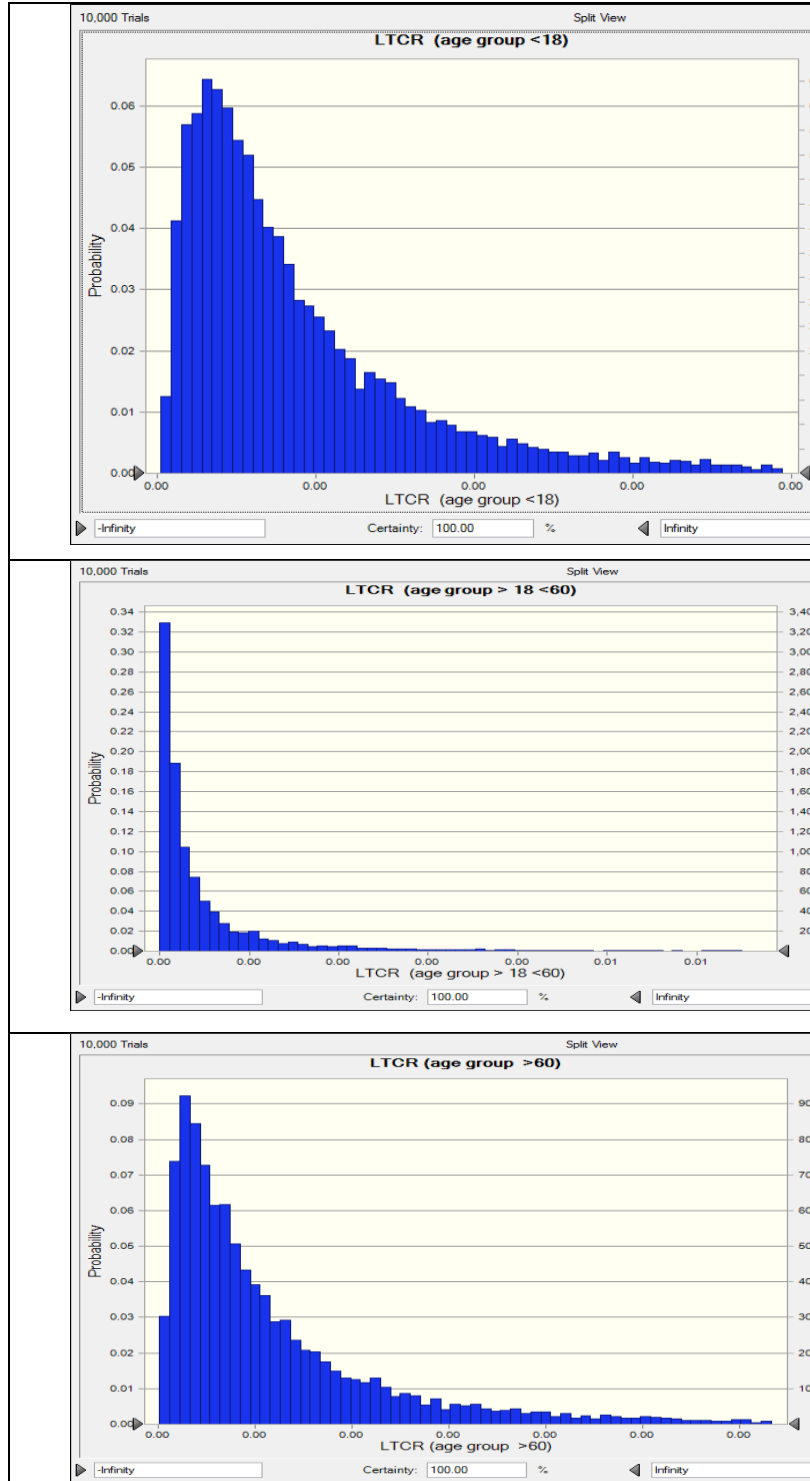
### 3.2.2. Carcinogenic risk

According to the results shown in Figure 5, for all of the samples in the all-age groups (age <18, age >18<60, and age > 60 years old), there is the cancer risk that indicates there was concern about carcinogenic risk due to acrylamide consumption through industrial popcorn with different flavours and brands. In this study, the rank order of LTCR for age group <18, >18<60, and >60 years were  $9.29\text{E-}4$ ,  $6.01\text{E-}4$ , and  $1.29\text{E-}4$ , respectively. According to the USEPA (2009), where the LTCR was higher than  $10^{-4}$ , there is the potential concern about people's health and acceptable safe level that is between  $10^{-4}$ - $10^{-6}$  (Fathabad et al., 2020). So, according to the LTCR of popcorn consumption for all-age

groups, AA concentration in this study has a positional risk for carcinogenic diseases. According to another study, AA has a carcinogenic risk for animals and human health, but another metabolite of AA, such as GA (glycinamide), is a hazardous and toxic effect rather than AA. So, type of food, cooking condition, time-heating, brands of food, and additives are the critical variables that affect AA concentration (Mollakhalili-Meybodi et al., 2021; Xu et al., 2014). Leilani et al. show that the AA level under cooking conditions and industrial or traditional methods and in the different nuggets (with chicken, meat, and shrimp) are different. Also, LTCR was safe for all samples (Seilani et al., 2021). In another study carried out by Eslamizad et al. on the

wheat flour of bread, similar to this study, all age group was at elevated carcinogenic risk (Eslamizad et al., 2019). While, in contrast to our study results, the result of a study in the

Romanian population shows that there was no cancer health risk for consumers (Oroian, Amariei, & Gutt, 2015b).



**Figure 5.** LTCR for different age groups that consumption the industrial popcorn.

Another study examined the dietary AA intake of Chileans for the first time and conducted risk evaluations using the margin of exposure (MOE) approach. MOEs for individuals aged 12 to 65 years were less than 10-4. According to the EFSA standards, there is a risk to public health. It is noteworthy that potatoes and bread provided around 77 percent of dietary acrylamide exposure in Chile, and children (12-17 years old) have the most significant dietary acrylamide consumption of all age groups (Barrios-Rodríguez et al., 2021), whose finding was consistent with the current study.

#### 4. Conclusions

This study detected AA concentration in popcorn products in the different brands with 5 flavours kind. The AA concentration in popcorn was calculated using HPLC (UV-DAD). Results show that the AA concentration in some popcorn brands that are produced in the domestic factories is high, so it needs to control and monitor AA mitigation in such popcorn products, which are used extensively by Iranian people, especially children aged 6-12 years old, till not to impose a health risk to humans. Also, health risk assessment in the three age groups showed that in all samples, the mean HQ value for AA intake was lower than 1, but it is notable, while the mean value of HQ was lower than 1, for part of populations age groups ranged from less than 18 years old and age groups higher than 18 and less than 60 years old, the HQ values was higher than 1, which indicated probable health risk for these population. Also, LTCR results for all consumers indicated the potential cancer risk. Therefore, control and monitoring of the different popcorn materials (oil, additives, and heat (°C) and production processes are essential.

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**Conflict of interest**

The authors declare no conflict of interest relevant to this article.

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Not applicable.

**Consent to Participate**

The authors declare their Consent to Participate in this article.

**Consent to Publish**

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**Authors Contributions**

Razieh Shahbazi, Behrouz Akbari Adergan, Khadijeh Jafari, Ensiyeh Taheri, Ayub Ebadi Fathabad, Naiema Vakili Saatloo: Investigation, Data curation, Resources, Conceptualization, Methodology, Writing, original draft. Nabi Shariatifar, Ebrahim Molaee Aghaee: Supervision, literature searching, writing & editing. Parisa Sadighara and Amin Mousavi Khaneghah: Supervision, review & editing.

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