ACRYLAMIDE CONTENT IN POPCORN FROM SPANISH MARKET: RISK ASSESSMENT

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Acrylamide content in popcorn from Spanish market: risk assessment

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Abstract

Snacks, including popcorn, are increasingly consumed in Spain and are susceptible to acrylamide (AA) formation. AA, classified as a probable human carcinogen by the International Agency for Research on Cancer (IARC), is produced via the Maillard reaction between reducing sugars and amino acids, particularly glucose, and asparagine, when foods are heated above 120°C. This study aims to analyze the AA content in 91 popcorn samples, categorized by flavor (salted, butter, caramel, flavored, colored, unflavored) and cooking method (ready-to-eat, popcorn maker, microwave), and assess dietary AA exposure in the Spanish population. Samples were collected from supermarkets, grocery stores, and cinemas across Spain and analyzed using solid-liquid extraction (SLE) and liquid chromatography-tandem mass spectrometry (LC-MS/MS). The average AA concentration in the samples was 277 ± 119 μg kg^{-1}, with only two samples below the limit of quantification (LOQ, 60 μg kg^{-1}). At the same time, no significant correlation between flavor and AA content was found. Whereas microwave cooking notably increased AA levels. Estimated AA intake for adults and children ranged from 0.011 to 0.045 μg kg^{-1} day^{-1}, depending on the exposure scenario. In children, a margin of exposure (MOE) below 10,000 was observed for Harderian gland tumors in realistic and pessimistic scenario.

Keywords: *Acrylamide, popcorn, dietary intake, risk assessment.*

1. Introduction

Zea mays everta is the only variety of corn whose kernel can pop into popcorn (Gopinath et al., 2024). This familiar cereal-based snack is consumed both at home and in movie theatres and its consumption has increased over the past few years in Spain (MAPA, 2021). On the other hand, popcorn is susceptible to acrylamide (AA) formation during cooking (Žilić et al., 2022).

AA is a toxic formed during Maillard reaction, occurring between reducing sugars and amino acids, especially glucose and asparagine, when foods are heated to more than 120 °C. The International Agency for Research on Cancer (IARC) classifies AA as a probable carcinogen (Hamzalıoğlu et al., 2019; IARC, 1994). The European Food Safety Authority (EFSA) concluded in 2015 that AA may be linked to an increased risk of developing cancers, including kidney, endometrial, and ovarian cancers, as well as potential interference with fetal development. Studies in animals have shown a connection between AA exposure and the development of genetic mutations, tumors, and adverse effects on the nervous system (EFSA, 2015). AA exhibits neurotoxic effects by damaging nerve fibers, impairing neurotransmitter release, and inducing oxidative stress in neurons. It can lead to peripheral neuropathy, characterized by muscle weakness, numbness, and motor dysfunction. Prolonged exposure may also disrupt central nervous system function, contributing to cognitive and behavioral deficits (Park et al., 2021).

Risk assessment is a critical process used to evaluate the potential health hazards posed by exposure to toxic substances such as AA. According to Joint FAO/WHO Expert Committee on Food Additives (JECFA) and EFSA, one key approach in this assessment is the application of the Margin of Exposure (MOE) concept. The MOE is calculated by comparing the level of AA exposure to the benchmark dose lower confidence limit ($BMDL_{10}$). The $BMDL_{10}$ is the level of exposure to AA with the dose known to cause harmful effects, typically in animal studies. An MOE of 10,000 or higher represents a low concern for public health regarding cancer risk (EFSA, 2005). A previous study suggests a health problem for the adolescent population related to AA and its consumption through popcorn (Akbari-Adergani et al., 2023).

To calculate exposure levels, it is first necessary to know the concentration of the contaminant in the food. Some of the foods most studied for their high levels of AA are coffee, potato products and bakery products (Sebastià et al., 2023). The Spanish Agency for Food Safety and Nutrition (AESAN) published a report in 2017 on AA levels in various food groups. According to the report, average AA concentrations were found to be 753 μg kg^{-1} in potato chips, 247 μg kg^{-1} in cookies, and 10 μg kg^{-1} in bread. These findings highlight significant variations in AA content across different food categories, which is influenced by cooking processes and ingredient composition (AESAN, 2017). The EFSA has provided comprehensive reports on the distribution of AA levels across various food categories; however, data specifically regarding popcorn and other corn-based snacks remain scarce (EFSA, 2015). In order to fill the gaps in literature, there is a need to report the AA content in popcorn. This is particularly important given the widespread consumption of popcorn and its potential to contribute significantly to overall dietary AA exposure. Understanding the AA levels in popcorn will provide valuable insights into public health risks and guide future regulatory measures aimed at reducing AA intake from such products.

Although there are no maximum limits for AA levels in food, in 2019, the Commission Regulation (EU) stresses the need to monitor AA levels in several foods such as churros, dried fruits and popcorn (Commission Regulation (EU) 2017/2158, 2017). Data on the AA content in popcorn comprises a high range between 0.5 and 14,951 μg kg^{-1} which may be due to cooking method. In addition, no current data are available regarding the AA content of popcorn commercialized in Spain (Sebastià et al., 2024). In the scientific literature, there are several studies evaluating the levels of AA in popcorn; however, none of these studies focus exclusively on this food matrix or provide a comprehensive overview of the presence of this food processing contaminant in popcorn.

The aim of the present study is to monitor the AA content in popcorn samples purchased in Spain from supermarkets, cinemas, grocery stores or through online shopping and to investigate the levels of AA in relation to different popcorn flavors and cooking methods. Moreover, an assessment of the dietary exposure of the Spanish population to AA through popcorn intake was performed.

2. Materials and methods

2.1. Reagents and Chemicals

Merck (Darmstadt, Germany) supplied high purity AA and AA-d_3 analytical standard (>99%) as solid. Stock solutions (1 mg L^{-1}) were prepared in deionized water. For the extraction procedure, deionized water with resistivity >18 MΩ cm^{-1} was obtained through Milli-Q SP® Reagent Water System (Millipore Corporation, Bedford, MA, USA). Zinc sulfate heptahydrate ($ZnSO_4 \cdot 7H_2O$) and magnesium oxide (MgO) were acquired from Thermo Fisher Scientific (Madrid, Spain). Formic acid was purchased from Thermo Fisher Scientific (Madrid, Spain). The liquid phase was filtered through VWR® Grade 302 filter paper, 8–12 μm (Darmstadt, Germany). Before the injection in the LC-MS/MS system, the extracts were filtered employing a 13-mm/0.22-μm nylon filter from Membrane Solutions (Plano, TX, USA)

The samples were homogenized using a Pulverisette 11 (Fritsch, Madrid, Spain). Centrifugation was carried out with an M-Universal centrifuge from MPW Med. Instruments (Warsaw, Poland). Water-based extracts were concentrated through nitrogen evaporation using a TurboVap LV (Zymark Corp., Hopkinton, MA, USA). Ultrasonication was performed in a Branson 5200 ultrasonic bath (Branson Ultrasonic Corp., Brookfield, CT, USA). The samples were stirred with a Vortex-Vib (J.P. SELECTA S.A., Barcelona, Spain) and shaken using a MultiMix Heat D magnetic stirrer (Ovan, Barcelona, Spain). Microwave popcorn was prepared using a Cm20lb microwave (Habitex), while stovetop popcorn was made with the Fun&Taste PCorn easy 200 W popcorn maker (Cecotec, Quart de Poblet, Spain).

2.2. Sample collection

A total of 91 popcorn samples were collected during 2024 from different supermarkets, grocery stores and movie theaters from Comunitat Valenciana (Spain) and some of them were purchased online. The samples were classified according to the flavor: butter, caramel, colored, flavored, salted and unflavored and to the cooking process: microwave, ready-to-eat (already popped) and popcorn maker. Although colored does not correspond to a flavor, it has been decided to separate it as a different category in order to determine if the presence of this ingredient can affect the AA content. Within the flavored group, all those flavors that are not commonly found on supermarket shelves, such as barbecue, cheese or spicy chili, have been grouped together. Table 1 shows the different categories of popcorn samples analyzed.

Table 1. Classification of samples according to the flavor and cooking process.

	Butter	Caramel	Colored	Flavored	Salted	Unflavoured	Total
Microwave	13	5	2	9	22	0	51
Ready-to-eat	0	5	4	3	8	0	20
Popcorn maker	0	0	0	0	0	20	20
Total	13	10	6	12	30	20	**91**

Stovetop popcorn samples were cooked in a popcorn popper for 2 min. Microwave popcorn samples were inserted into the microwave oven and heated following the manufacturer's guidelines (3 min at 700 W). The excessively burned popcorn, which was not suitable for consumption, as well as the unpopped kernels, were discarded for the analysis.

The sampling procedure was based on the methodology described in the annex B of the European Commission Regulation (EC) 333/2007 (European Commission, 2007).

2.3. Sample preparation

The samples´ extraction was carried out by solid-liquid extraction (SLE) followed by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) according to the method proposed in a previous work (Sebastià et al., 2024). Before the extraction, 1 kg of sample was ground into powder. Subsequently, 333 µL of a 1 mg L^{-1} AA-d_3 solution, used as internal standard (IS), was added to 1 g of the homogenized sample. After 15 min, 10 mL of Mili-Q water was added, and the resulting mixture was ultrasonicated (20 kHz frequency and 100 W power) and then shacked for 10 min. The mixture was centrifuged 10 min at 2504 x *g*, the liquid phase was filtered and transferred into a 15 mL centrifuge tube. To clean-up the extract, MgO (0.25 g) and $ZnSO_4 \cdot 7H_2O$ (0.25 g) were added to a 3.5 mL fraction of the aqueous phase and the mixture was vortexed for 1 min. After 10 min centrifugation at 2504 x *g*, the phase separation was achieved, and the solid phase was discarded. An aliquot of 1.5 mL from the liquid phase was transferred into a 5 mL tube. Then, the extract was evaporated to dryness under nitrogen flow and the residue was reconstituted with 0.5 mL of Mili-Q water and vortexed for 1 min. Finally, the extract was centrifuged (2504 x *g*, 10 min) to discard the residual starch and filtered through a 0.22 µm nylon filter prior to the injection in the LC-MS/MS system.

2.4. Acrylamide determination

AA analysis was carried out using an Agilent 1100 series chromatograph (Agilent Technologies, Palo Alto, CA, USA) coupled to a Finnigan (Waltham, MA, USA) TSQ Quantum Ultra mass spectrometer system. The separation of analytes was conducted using an Aquasil C18 column (100 × 2.1 mm, 3 μm particle size) supplied by Thermo Scientific (Waltham, MA, USA).

Five microliters of standards or sample extracts were injected into LC-MS/MS working in isocratic mode with a mixture 0.1% formic acid in Mili-Q water as mobile phase at 0.2 mL min^{-1}. MS/MS acquisitions were performed using multiple reaction monitoring (MRM) using an atmospheric pressure chemical ionization (APCI) source in positive ion mode.

A discharge current of 3 μA, a vaporizer temperature of 250 °C, and a capillary temperature of 350 °C were set in the source. Nitrogen gas, produced by a Zefiro 35 LC-MS nitrogen generator (Cinel Gas Generators S.R.L., Vigonza, Italy), was used as sheath gas (25 psi) and auxiliary gas (3 psi) for nebulization and desolvation, respectively. Argon was employed as collision gas for collision induced dissociation at a pressure of 1.0 mTorr. The employed MRM transitions were 72>44 and 72>55 m/z for AA, and 75>44 and 72>58 m/z for AA-d_3. Collision energies of 24 and 11 V were employed for the first and second transitions, respectively. Sample processing was performed using TSQ Series 2.3 SP3 software from Thermo Fisher Scientific. Data treatment was carried out by Xcalibur software from Thermo Fisher Scientific.

2.5. Quality control/quality assurance

The sample preparation involved the use of SLE and a clean-up procedure with dSPE, followed by analysis through LC-MS/MS, as described in a previous study (Sebastià et al., 2024). The proposed method provided quantitative recoveries (100–101%), and a high precision with relative standard deviation lower than 6%). The limit of detection (LOD) and the LOQ for popcorn were 17 and 60 μg kg^{-1}, respectively. The matrix effect was non-significant (2%); so, the use of matched matrix standards was not required. Quantification of AA was achieved using a calibration curve in water, with concentrations ranging from 5 to 1000 μg L^{-1} of AA, including 100 μg L^{-1} of AA-d_3 as an IS. To ensure accuracy, each batch of samples included a blank, which was processed identically to the test samples to assess potential AA contamination. Additionally, quality control was ensured through analyzing

spiked samples every two analytical sets. External quality assurance was performed using FAPAS 30114 (cookie), yielding satisfactory results with |z-scores| ≤ 2.

2.6. Statistical analysis

All analyses and procedures were carried out in triplicate. Statistical analyses were performed by using GraphPad 9 (GraphPad Software, San Diego, CA, USA). For determining the significant differences among samples was applied One-way analysis of variance (ANOVA). For the statistical a p-value < 0.05 was considered statistically significant.

3. Results and discussion

3.1. Acrylamide content in popcorn samples

The incidence of AA in popcorn samples (n = 91) was 100%, with a mean concentration of 277 ± 119 μg kg^{-1}. Only in two samples the AA content was below the LOQ (60 μg kg^{-1}) and none of them presented values below the LOD (17 μg kg^{-1}). The relatively high mean and the large standard deviation suggest that while AA is present in all samples, there is a substantial variation in its concentration depending on the flavor and preparation method (Table 2). The quartiles (Q1 and Q3) show that most of the samples (50%) had AA concentrations between 192 and 360 μg kg^{-1}, a range that can be considered typical for most samples.

Table 2. Descriptive analysis for acrylamide content in popcorn samples grouped according to flavor and cooking process. Data expressed in μg kg^{-1}.

	Butter	Caramel	Colored	Flavored	Salted	Unflavored	Microwave	Popcorn maker	Ready-to-eat	Total
n	13	10	6	12	30	20	51	20	20	91
Mean	318	260	214	266	314	228	315	228	229	277
SD	114	145	112	126	124	73	100	73	163	119
Min	88	88	96	< LOQ	< LOQ	112	85	112	< LOQ	< LOQ
Max	529	506	418	453	627	393	529	393	627	627
Q1	249	112	134	184	232	167	250	167	93	192
Median	335	259	188	302	331	214	318	214	196	271
Q3	388	361	291	344	394	271	390	271	349	360
P90	491	500	418	437	460	364	436	364	462	432

SD: Standard deviation. Min: Minium. Max: Maximum. Q1: First quartile. Q3: Third quartile. P90: 90th percentile. LOQ: Limit of quantification.

No statistically significant differences (p-value < 0.05) were observed in AA contents when comparing the total samples based on flavor (Figure 1). However, when grouping the samples by cooking method (Figure 2a), it was observed that microwave-cooked samples showed a difference in AA content compared to those cooked in popcorn maker and the ready-to-eat samples.

Although it might be expected that the presence of added sugars in caramel popcorn would have influenced the final content of AA, the results obtained in this study did not support these expectations. It could be attributed to the fact that the sugars present in the product are unable to interact with the proteins in the corn kernel, as the kernel is covered by the pericarp (outermost layer) (Casas et al., 2014). However, as noted by other researchers, the cooking method is likely the most significant factor affecting the AA content (Hu et al., 2017; Pacetti et al., 2015; Zhang et al., 2008).

The susceptor contained in the package for microwave cooking, converts the electromagnetic energy into thermal energy causing the corn to pop (Roach et al., 2003), this susceptor does not distribute the heat evenly throughout the package, which may cause overheating in some areas of the bag and, consequently, a higher AA content in the popped popcorn. In contrast, popcorn cooked in domestic popcorn makers and ready-to-eat samples, which are prepared in larger capacity popcorn makers, experience more uniform heat, preventing the additional generation of AA once they are cooked. However, no significant differences were observed in the AA content of microwave-cooked popcorn based on flavor type (Figure 2b).

Benchmark levels, although not directly related to health, give an approximate idea of the usual content of a substance in a type of food (AESAN, 2020). There is currently no established benchmark level for AA in popcorn or corn-based snacks. However, in this study, the benchmark level for maize-based breakfast cereals has been used as a reference, which is set at 150 μg kg^{-1} (Commission Regulation (EU) 2017/2158, 2017). The 87% of the samples analyzed exceed this reference limit. This suggests that it is required to monitor the amount of AA in these products and assess whether it is necessary to establish a benchmark level for AA in popcorn.

In 2017, the EC introduced a set of mitigation measures aimed at reducing the presence of AA, in various food products. These regulations primarily targeted foodstuff such as potatoes, bread, and breakfast cereals. Despite the broad scope of these measures, no specific guidelines were provided for cereal-based snacks, and notably, popcorn (Commission Regulation (EU) 2017/2158, 2017).

For breakfast cereals, the reduction of AA content follows the ALARA principle (As Low As Reasonably Achievable). This principle is widely used in food safety and risk management, mandating that AA levels be minimized as much as feasible possible, taking into account economic and technological constraints. The goal is to lower exposure to AA without compromising product quality or availability (FoodDrinkEurope, 2019).

3.2. Risk assessment of acrylamide through popcorn consumption

The estimated daily intake (EDI) of AA through popcorn consumption was calculated employing Eq. 1:

$$EDI = \frac{Ci \; x \; Ii}{BW} \quad (1)$$

Ci: concentration of acrylamide in popcorn (μg kg^{-1})
Ei: daily average consumption of popcorn (kg $person^{-1}$ day^{-1})
BW: body weight (kg)

The consumption data of popcorn in Spain has been obtained from "Panel de consumo alimentario (PCA) of Spain" and "the Encuesta Nacional de Alimentación en la población Infantil y Adolescente (ENALIA)" as well as "the Encuesta Nacional de Alimentación en población adulta, mayores y embarazadas" (ENALIA 2) (AESAN, 2015a, 2015b; MAPA, 2021).

The main limitation of this study was the challenge to obtain precise data on popcorn consumption, as it is typically grouped under the category of snacks in dietary surveys. Additionally, a distinction should be made between the average consumption for the general population and the average consumption among regular consumers. In this study, based on the information provided by the questionnaires and to ensure a representative estimate, popcorn consumption in adults was estimated at 2.9 g $person^{-1}$ day^{-1}, roughly equivalent to one bag of popcorn (90 g) per month. In the child population, consumption was slightly higher, at 3.3 g $person^{-1}$ day^{-1}.

To estimate the dietary AA exposure from popcorn, two different scenarios were considered. In the first, a realistic approach was applied, utilizing the average AA content across the samples. In contrast, the second scenario adopted a more conservative, or pessimistic, perspective, where P95 (463 μg kg^{-1}) was used to calculate potential exposure. These approaches aim to provide a comprehensive assessment of AA intake under typical and worst-case conditions.

The dietary intake of AA in Spain for an adult population (70 kg) and child population (30 kg) from the popcorn samples analyzed in our study was estimated to approximately 0.011 and 0.03 μg kg^{-1} day^{-1}, respectively in the realistic scenario (RS) and 0.019 and 0.045 μg kg^{-1} day^{-1}, respectively in the pessimistic scenario (PS).

In 2015, the EFSA estimated that the median AA intake for adults was 0.5 μg kg^{-1} day^{-1}, while for children it was 1.3 μg kg^{-1} day^{-1}. The more common dietary sources of AA are fried, deep-fried, or baked food products, such as potatoes, bakery products and coffee. (EFSA, 2015). Taking into account this data, the AA intake from popcorn consumption in adults would account for 2.2% in a RS and 3.8% in a PS, whereas the corresponding intake would be 2.3% and 3.5%, respectively, for children. Similar to the data estimated by EFSA, a higher AA intake is also observed in children in this study. It is important to emphasize that popcorn is just one food item contributing to overall dietary AA exposure, combined with other dietary sources, occupational exposure, or more commonly, tobacco use.

The MOE was employed to assess the most sensitive carcinogenic effect of AA (Mesías & Morales, 2015) employing Eq. 2. The $BMDL_{10}$ provided by EFSA for mammary tumors in female rats is 310 μg kg^{-1} day^{-1} and for Harderian gland tumors in male mice is 180 μg kg^{-1} day^{-1} (EFSA, 2015). The Scientific Committee of EFSA claims that an MOE of 10,000 or greater is considered to pose minimal concern from a public health perspective regarding carcinogenic risks. Since a lower MOE indicates a higher risk, it follows that a significantly elevated MOE would be highly unlikely to raise safety concerns. Thus, high MOE provides a substantial margin, suggesting that exposure levels are far from those that could potentially lead to adverse health effects (EFSA, 2012).

$$MOE = \frac{BMDL10}{EDI} \quad (2)$$

MOE: margin of exposure
BMDL10: benchmark dose lower confidence limit
EDI: estimated daily intake

The MOE obtained from the data provided in this study is presented in **Table 3.** In a RS, an MOE less than 10,000 has been determined for Harderian gland tumors in the child population. In contrast, in a PS, an MOE of less than 10,000 is observed for both mammary tumors in the adult population and for Harderian gland tumors in both populations.

Table 3. Acrylamide dietary exposure and margin of exposure obtained from data supplied by this study.

	Realistic scenario			**Pessimistic scenario**		
	EDI ($\mu g\ kg^{-1}\ day^{-1}$)	MOE for MT	MOE for HGT	EDI ($\mu g\ kg^{-1}\ day^{-1}$)	MOE for MT	MOE for HGT
Adult population	0.011	28,181	16,363	0.019	16,316	9,474
Child population	0.03	10,333	6,000	0.045	6,889	4000

EDI: Estimate daily intake. MOE: Margin of exposure. MT: Mammary tumours in female rats. HGT: Harderian gland tumours in male mice

In the scientific literature, numerous studies have conducted risk assessments to evaluate AA exposure through various food products, consistently reporting similar outcomes. Mesias & Morales 2015 assessed AA exposure from potato crisps, a widely consumed snack, within the Spanish population, reporting MOE values of 8,857 for mammary tumors in female rats and 5,143 for Harderian gland tumors in male mice (Mesías & Morales, 2015).

In another study, El Tawila et al. 2017 assessed AA exposure from cafeteria foods in Jeddah schools, yielding MOE values below 500 for both tumor types under both realistic and pessimistic (P95) exposure scenarios (El Tawila et al., 2017).

Similarly, Yu et al. 2023 performed a dietary exposure assessment of AA in Singapore, focusing on foods consumed both within and outside of main meals. Their findings revealed MOE values below 10,000 among high AA consumers (P95) and values near 10,000 for the general population, indicating potential risks for frequent consumers (Yu et al., 2023).

Additionally, Pardo and colleages conducted a comprehensive risk assessment of AA exposure in Spanish adults, children, and lactating mothers using human biomonitoring techniques. The EDI in this study was based on AA metabolites detected in urine samples, offering a more direct measurement of exposure across various population groups. This approach, in contrast to previous studies, provided a more holistic dietary exposure

assessment across multiple food sources (Fernández et al., 2022a, 2022b; Peris-Camarasa et al., 2023). All studies, in line with the current assessment, conclude that exposure to AA represents a major public health problem through both popcorn and other snacks such as potato chips.

3.3. Comparison with other studies

In the present study, a high degree of variability in AA content was observed across popcorn samples. This extensive variability is reflected in the range of the samples, as well as in the standard deviation and coefficient of variation (43%) as shown in Table 4. Similar findings have been reported in other studies, including those by (Bušová et al., 2020; El Tawila et al., 2017; Murkovic, 2004), which also noted significant differences between samples.

The AA concentrations in the samples analyzed in this study are comparable to those reported in previous research (Table 4). Notably, most studies focus on microwave-cooked popcorn, where the average AA concentration found in this study has been around 315 $\mu g\ kg^{-1}$.

As observed in Table 4, most studies report a limited sample size ($n < 20$) and primarily focus on a single category of popcorn, specifically microwave varieties. This narrow scope complicates the accurate determination of AA levels across different popcorn products. In contrast, our study utilizes a more representative sample size ($n = 91$), incorporating a range of cooking methods and flavour profiles. Despite these methodological differences, the AA concentrations observed in our study are consistent with those reported in previous research.

Table 4. Acrylamide mean levels found in popcorn samples in different studies.

Food matrix	Country	n	Mean ± SD (µg kg^{-1})	Range (µg kg^{-1})	Reference
Traditional popcorn	Iran	12	7700	2411 - 14951	(Akbari-Adergani et al., 2023)
Industrial popcorn	Iran	32	3080	LOD - 10283	
Popcorn and cereal-based snack bars	Singapore	14	127 ± 32	0.5 - 375	(Yu et al., 2023)
Popcorn	Iran	3	218	162 - 259	(Kamankesh et al., 2021)
Popcorn	Czech Republic	8	761 ± 304	433 - 1410	(Bušová et al., 2020)
Popcorn	Saudi Arabia	7	171	80 - 269	(Khan et al., 2018)
Popcorn	Saudi Arabia	20	150 ± 68	74 - 235	(El Tawila et al., 2017)
Popcorns and cornflakes	China	14	524 ± 187	18 - 1966	(Hu et al., 2017)
Popcorn	Colombia	5	452	< LOQ - 781	(Pacetti et al., 2015)
Popcorn	Canada	4	329	213 - 457	(Normandin et al., 2013)
Popcorn	Finland	3	300 ± 46	260 - 350	(Eerola et al., 2007)
Popcorn and rice products	Austria	15	106 ± 114	< LOQ - 308	(Murkovic, 2004)
Popcorn	Sweden	3	500	365 - 715	(Svensson et al., 2003)
Popcorn	United States	2	169	157-181	(Roach et al., 2003)
Popcorn	Spain	91	277 ± 119	< LOQ - 627	This study

SD: Standard deviation. LOD: Limit of detection. LOQ: Limit of quantification.

Akbari-Adergani et al. 2023 reported the most divergent data in comparison to our study and those presented in the table (Akbari-Adergani et al., 2023). This study collected samples of popcorn prepared through industrial and traditional (where temperature control is less regulated) cooking methods. Similar to our findings, this research also demonstrates that the cooking method significantly impacts the amount of AA formed.

4. Conclusions

This study represents the most comprehensive analysis of AA content in popcorn samples to date and is the first to systematically monitor AA levels based on cooking method and flavoring. The findings emphasize the critical role of the cooking method, with microwave popcorn emerging as the most significant contributor to elevated AA levels. The risk assessment conducted in this study seems to indicate a concern about AA exposure in children in a pessimistic scenario. Given the potential health risks, especially for vulnerable populations, this study highlights the need for stricter monitoring and regulatory controls in

line with EC recommendations to reduce AA levels in commercially available popcorn products.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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