

Occurrence, dietary exposure, and toxicological insights into acrylamide contamination in bakery products in Bangladesh

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Abstract

Acrylamide, a potential carcinogen and neurotoxin, forms in carbohydrate-rich foods during high-temperature cooking processes like baking. Despite global concerns, limited data exist on acrylamide levels in bakery products in Bangladesh, where dietary habits and processing methods may differ. This study aimed to quantify acrylamide levels in commonly consumed bakery products (bread, cake, burger bun, and pizza) in Bangladesh, assess dietary exposure, and evaluate associated health risks. Thirty-six samples were collected from various regions and analyzed using gas chromatography with electron capture detection (GC-ECD). Risk assessment was conducted using the Margin of Exposure (MOE) approach for neurotoxicity and carcinogenicity. Acrylamide contamination was widespread, with 75% of bread, 100% of cakes, 83% of burger buns, and 83% of pizza samples testing positive. Notable exceedances of benchmark levels were observed in bread (67%), cakes (33%), and burger buns (80%). Burger buns exhibited the highest dietary acrylamide exposure (up to $4.284 \mu\text{gkg}^{-1}$ body weight per day), while pizza showed the lowest ($0.025 \leq \mu\text{gkg}^{-1}$ body weight per day). Risk assessment revealed significant neurotoxic ($\text{MOE}_n < 100$) and carcinogenic ($\text{MOE}_c < 10,000$) risks for certain products, particularly burger buns and bread. The findings highlight the pervasive nature of acrylamide in bakery products, driven by high-temperature processing. Variations in contamination levels across regions and products underscore the need for optimized baking conditions and mitigation strategies. Stricter regulatory guidelines, improved processing techniques, and public awareness campaigns are essential to reduce acrylamide exposure. Continuous monitoring and research are recommended to address regional variations and ensure food safety.

1. Introduction

Acrylamide ($\text{C}_3\text{H}_5\text{NO}$) is a low-molecular-weight organic compound that has garnered significant attention due to its presence in thermally processed foods (Amanda and House, 2024). It is classified as a potential carcinogen and neurotoxin, raising concerns about its impact on human health (Merhi *et al.*, 2020; Sarion *et al.*, 2021). Acrylamide is not naturally present in raw foods but forms during high-temperature cooking processes such as frying, baking, roasting, and toasting, typically at temperatures above 120°C (Simões de Borba *et al.*, 2023; Adimas *et al.*, 2024; Pandiselvam *et al.*, 2024). The primary mechanism of acrylamide formation is the Maillard reaction, a non-enzymatic browning process that occurs between the amino acid asparagine and reducing sugars (e.g., glucose and fructose) in carbohydrate-rich foods (Liu *et al.*, 2022; Govindaraju *et al.*, 2024; Oliver *et al.*, 2024; Stadler and Gökmen, 2024). This reaction not only contributes to the desirable

flavor, color, and texture of baked goods but also leads to the unintended formation of acrylamide as a byproduct (Augustine and Bent, 2022; The Maillard reaction, 2024).

Bakery items, such as bread, cakes, burger buns, and pizza, are particularly susceptible to acrylamide formation due to their high carbohydrate content and the baking process involved in their preparation (Ahmad *et al.*, 2022; Çebi, 2024). Studies have shown that the acrylamide content in these products varies depending on factors such as cooking time, temperature, and the composition of raw ingredients (Sarion *et al.*, 2021; Schouten *et al.*, 2022). For instance, crusts of bread and pizza, which are exposed to higher temperatures, often exhibit elevated acrylamide levels compared to their inner portions (Ahrné *et al.*, 2007; Rose *et al.*, 2023). Understanding and reducing acrylamide formation in widely consumed bakery products is crucial for food safety and public health, as it is classified by IARC as a

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probable human carcinogen (Group 2A) due to its toxicological effects (Sarion *et al.*, 2021; Başaran *et al.*, 2023). Epidemiological studies suggest a possible link between dietary acrylamide exposure and an increased risk of cancers, including kidney, ovarian, and endometrial cancers (Filippini *et al.*, 2022; Quartey *et al.*, 2024). Beyond carcinogenicity, acrylamide is associated with neurotoxicity and reproductive toxicity (Lindeman *et al.*, 2021). Animal models have shown that acrylamide exposure can lead to peripheral nerve damage, motor dysfunction, and cognitive impairments (Wang *et al.*, 2022; Zhao *et al.*, 2022; Rajeh, 2024). Additionally, studies indicate adverse effects on reproductive health, including reduced fertility and developmental toxicity in offspring (Zhang *et al.*, 2022; Gupta *et al.*, 2023). These findings underscore the need for caution, particularly for vulnerable populations such as pregnant women and children.

Given the widespread consumption of acrylamide-containing foods, such as bakery products, monitoring its levels in the diet is critical for public health. Regulatory agencies, including the European Food Safety Authority (EFSA), have emphasized the importance of reducing dietary acrylamide exposure to mitigate potential health risks (EFSA, 2011; Mihalache and Dall'Asta, 2024). This highlights the urgency of ongoing research and surveillance to ensure food safety and protect consumers. Bakery items, such as bread, cakes, burger buns, and pizza, are of particular concern due to their global consumption and the high-temperature processing involved in their preparation. These products are carbohydrate-rich, containing asparagine and reducing sugars, which are precursors for acrylamide formation during baking or frying (Rifai and Saleh, 2020; Henao Toro *et al.*, 2022; Adimas *et al.*, 2024; Batuwita *et al.*, 2024). Global studies have reported acrylamide concentrations in bakery products ranging from 20 to 500 $\mu\text{g}/\text{kg}$, with higher levels found in well-browned or toasted items (Khan *et al.*, 2019; Verma and Yadav, 2022; Perestrelo *et al.*, 2024). For example, bread samples from Europe and Asia have shown acrylamide levels averaging 50–200 $\mu\text{g}/\text{kg}$, while cakes and biscuits often exceed 300 $\mu\text{g}/\text{kg}$ (EFSA, 2011; Scientific Opinion on acrylamide in food, 2015; Maher *et al.*, 2022; Zhuang *et al.*, 2022; Fan *et al.*, 2023). These findings highlight the need for localized studies in regions like Bangladesh, where dietary habits and processing methods may differ. Globally, regulatory bodies have established guidelines to mitigate acrylamide levels in food due to its potential health risks. The European Commission has set benchmark levels for acrylamide in various food categories, including bakery products, with bread and biscuits limited to 50–350 $\mu\text{g}/\text{kg}$ (EU, 2017; Sarion *et al.*, 2021). Similarly, the U.S. FDA has issued

guidance for food manufacturers to reduce acrylamide through process optimization, though no mandatory limits are in place (FDA, 2016). The World Health Organization (WHO) also emphasizes the need for continuous monitoring and risk assessment of acrylamide in the diet (WHO, 2002; FAO/WHO, 2002; WHO Food Safety Programme, 2002).

Acrylamide detection in food matrices relies on advanced analytical techniques, including gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), and gas chromatography with electron capture detection (GC-ECD) (Nemoto *et al.*, 2002; Dunovská *et al.*, 2006; Pundir *et al.*, 2019; Skinner *et al.*, 2021). GC-MS and LC-MS are widely used for their high sensitivity and ability to quantify acrylamide at trace levels (Hasan *et al.*, 2022; Gökmen, 2024; Sun *et al.*, 2023). However, GC-ECD offers a cost-effective and reliable alternative, particularly for laboratories with limited resources (Zhu *et al.*, 2008; Oracz *et al.*, 2011; Pundir *et al.*, 2019; Bertuzzi *et al.*, 2020; Perera *et al.*, 2021). In this study, GC-ECD was chosen for its robustness, affordability, and ability to deliver precise results, aligning with the need for accessible methods in regions like Bangladesh. Despite the global concern over acrylamide in food, there is a significant lack of studies focusing on acrylamide levels in bakery products in Bangladesh and similar regions. This study quantifies acrylamide levels in commonly consumed bakery items in Bangladesh, including bread, cakes, burger buns, and pizza, due to their high-temperature processing. Using GC-ECD, the study provides the first comprehensive local assessment of acrylamide concentrations. The findings will inform public health policies, regulatory frameworks, and industry practices to minimize acrylamide formation. By raising awareness among consumers, manufacturers, and policymakers, the research contributes to food safety and public health efforts in Bangladesh.

2. Materials and methods

2.1 Glassware, chemicals, and equipment

Various laboratory apparatus and instruments were utilized in this research. The glass and plastic ware included round-bottom flasks (100 mL), conical flasks (100 mL), graduated test tubes, graduated pipettes (1.0 and 25.0 mL), Pasteur pipettes, micro pipettes, volumetric flasks (10.0-250.0 mL), mortars and pestles, beakers, GC vials, spatulas, and zip-lock plastic bags for sampling. All glassware was cleaned with detergent and water, rinsed thoroughly, followed by distilled water and acetone, then oven-dried at 102°C and stored under aluminum foil. Analytical and reagent-grade chemicals included KBrO_3 (SMART LAB, Indonesia), 12 M HCl (BDH, UK), and Acrylamide (>99.8%, Sigma-Aldrich,

Table 1. Sampling information of different baked goods of Bangladesh.

Bread	Sampling stations	Cake	Sampling stations	Burger bun	Sampling stations	Pizza	Sampling stations
B-1	Narsingdi	C-1	Mirpur-1	BB-1	Mirpur-1	P-1	Dhanmondi
B-2	Narayanganj	C-2	Lalmatia	BB-2	Dhanmondi	P-2	Lalbagh
B-3	Faridpur	C-3	Shyamoli	BB-3	Kazipara	P-3	Kotwali
B-4	Mymensingh	C-4	Savar	BB-4	Lalbagh	P-4	Motijheel
B-5	Sylhet	C-5	Patuakhali	BB-5	Mirpur-10	P-5	Azimpur
B-6	Rajshahi	C-6	Khulna	BB-6	Mohammadpur	P-6	Shyamoli
B-7	Jessore	C-7	Tejgaon				
B-8	Noakhali	C-8	Joypurhaat				
B-9	Patuakhali	C-9	Pabna				
B-10	Barisal	C-10	Panchagarh				
B-11	Bandarban	C-11	Barishal				
B-12	Bhola	C-12	Comilla				

USA) was used for standard solutions. Other chemicals included NaCl (Sigma-Aldrich), H₂SO₄ (98%, BDH, UK), KBr (Merck, Germany), Na₂S₂O₃ (Scharlau, Germany), Na₂SO₄ (Merck, Germany), n-hexane (Merck, Germany), and ethyl acetate (RCI Labscan, USA). Distilled and deionized water was obtained from a Milli-Q system. Instruments used were a Zeeman atomic absorption spectrometer (Varian, Australia), UV spectrophotometer (Shimadzu UV-1800), GC-ECD (Shimadzu-2030, Japan), analytical balances, an oven, a Carbolite furnace, a kitchen blender, a rotary vacuum evaporator, a centrifuge, and a vortex machine.

2.2 Sampling area and sampling

The study region of the research work was intended to be the entirety of the country. The sampling methodology included about thirty-six different sampling stations of Bangladesh. In total, thirty-six samples were collected from various bakeries, restaurants, and fast-food places in Dhaka and different districts of Bangladesh. There were 12 breads (B), 12 cake (C), 6 burger buns (BB), and 6 pizza (P) samples. The choice of these samples was based on the highest consumption and popularity among the populace in each location. The cake samples were coded as shown in the Table 1. The samples were gathered at the beginning of the days and immediately taken to the laboratory for analysis. All samples were acquired and analyzed within the recommended time of consumption.

2.2.1 Sample pretreatment and storage

Approximately 10-12 g of each sample was collected from its center and dried at 75°C in an oven for 2 hours. Due to the greasy nature of cakes and the presence of cheese, meat, and various toppings in pizza, the final extract for spectrophotometric analysis appeared turbid. To eliminate this turbidity, a defatting process was performed by heating the samples with n-hexane in a water bath, followed by oven drying. Similarly, the turbidity observed in burger buns resulted from the oily patty, cheese, and other fillings. To ensure thorough defatting, the powdered bun samples underwent pretreatment with n-hexane and were subsequently dried. After drying,

all samples were finely pulverized using a grinder, sealed in zip-lock bags, and stored under refrigeration for further analysis. This rigorous sample preparation ensured the removal of interfering lipids, facilitating accurate spectrophotometric measurements.

2.3 Determination of acrylamide

The acrylamide content in the samples was determined using gas chromatography equipped with an electron capture detector (GC-ECD) (Zhang *et al.*, 2006; Zhu *et al.*, 2008). Acrylamide is a highly polar, non-volatile compound with poor retention time and peak shape in conventional non-polar or weakly polar GC columns. Therefore, derivatization is essential to enhance its volatility for effective GC separation (Agilent technologies, 2014). This process involves bromination using KBrO₃ and KBr, yielding two derivatives: 2,3-dibromopropionamide (2,3-DBPA, <5%) and 2-bromopropenamide (2-BPA, >95%). These derivatives exhibit superior GC properties, including sharp peaks and high ECD response, and are significantly less polar than acrylamide, making them readily soluble in non-polar solvents such as ethyl acetate and n-hexane. Among the two, 2-BPA was selected as the quantitative analyte due to its peak response being nearly 20 times higher than that of 2,3-DBPA, ensuring enhanced sensitivity and accuracy in acrylamide quantification (Zhang *et al.*, 2006; Zhu *et al.*, 2008; Prost, 2010).

2.4 Standard solution of acrylamide and method performance

A stock solution of acrylamide (10 µg mL⁻¹) was prepared by dissolving acrylamide in distilled water. Aliquots of 10 µL, 25 µL, 50 µL, 100 µL, and 200 µL from this primary stock solution were transferred into 10.0 mL volumetric flasks and diluted to volume with distilled water, yielding standard solutions of 10 ppb, 25 ppb, 50 ppb, 100 ppb, and 200 ppb, respectively. The prepared solutions were then transferred to glass tubes, followed by the addition of 0.6 mL of 10% (v/v) H₂SO₄, and refrigerated at 4°C for 15 minutes. For derivatization, 1 mL of 0.1 M KBrO₃ and 1.5 g of KBr were added to each tube. The mixtures were vortexed

and allowed to stand at 4°C for 30 minutes. The reaction was then quenched by adding 0.1 mL of 0.1 M Na₂S₂O₃. A 4 mL aliquot of the analyte solution was transferred into a separatory funnel and extracted three times with 4 mL of redistilled ethyl acetate. The combined extracts were evaporated to dryness using a rotary vacuum evaporator. Subsequently, 4 mL of *n*-hexane was added to the dried residue, and the solution was ultrasonicated for 5 minutes. The *n*-hexane extract was then filtered through cotton using a Pasteur pipette over anhydrous sodium sulfate to remove residual moisture before being collected in a GC vial for analysis by GC-ECD.

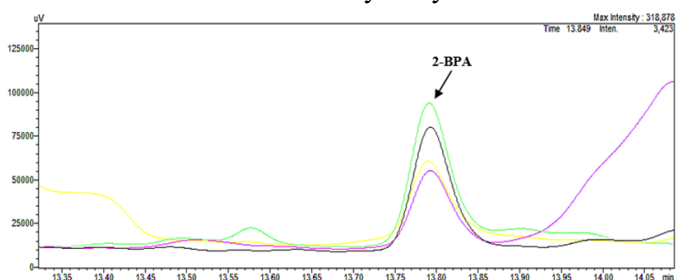


Figure 1. Overlain chromatogram of standard solutions of acrylamide.

The peak areas corresponding to acrylamide concentrations (Figure 1), with a retention time of 13.79 minutes, were used to construct a calibration curve. The regression equation of the calibration plot was determined using the least squares method for quantifying acrylamide in the samples. A calibration curve was first constructed to determine acrylamide levels in the samples. Acrylamide concentrations were calculated from their corresponding peak areas using the calibration equation: $y = 2061.6x + 29671$, where y represents the peak area and x denotes the concentration (Figure 2). This equation enabled accurate quantification of acrylamide in various samples, ensuring precise analytical evaluation based on the plotted calibration curve.

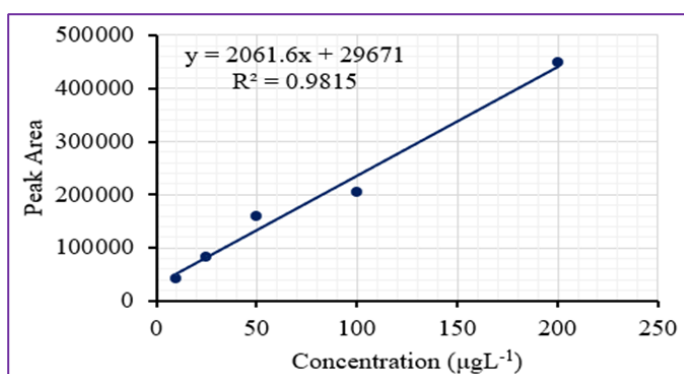


Figure 2. Calibration curve of standard acrylamide.

The method performance was evaluated through recovery experiments by spiking the standard solution with real samples. It included eight different samples, to assess the accuracy and precision of the current method employed for the determination of acrylamide. To 1.5 g of each sample measured, 500 µL of 1 µg mL⁻¹ of

bromate was added to spike the samples. For about ten minutes, the sample matrixes were left to stand. As previously described, acrylamide analysis was performed on each sample. The following formula was utilized for the determination of the recovery (R) percentage.

$$\text{Recovery (\%)} =$$

$$\frac{\text{Conc. of acrylamide in spiked sample} - \text{Conc. of acrylamide in unspiked sample}}{\text{Conc. of acrylamid added in the sample}} \times 100$$

The limit of detection (LOD) and limit of quantification (LOQ) were calculated using the following equations (Shrivastava and Gupta, 2011).

$$\text{LOD} = (3.3/b) \times S$$

$$\text{LOQ} = (10/b) \times S$$

Where, S = Standard deviation of intercept of the plot of peak area vs. acrylamide conc. of the samples b = Slope of the plot of peak area vs. acrylamide conc. of the samples.

2.5 Sample preparation

A precisely weighed 1.5 g portion of the powdered sample was transferred into a centrifuge tube. To remove lipids, 20 mL of redistilled *n*-hexane was added, followed by vortex mixing and ultrasonic shaking for 10 minutes. The supernatant *n*-hexane was discarded, and the defatting process was repeated. The residue was then collected for acrylamide extraction. For analyte extraction, 7 mL of 2 M NaCl was added to the residue, and the tube was shaken in an ultrasonic shaker. The mixture was then centrifuged at 4000 rpm for 15 minutes, and the clarified aqueous layer was quickly removed using a pipette. This extraction step was repeated, and the supernatants from both extractions were combined for further analysis. A 5 mL aliquot of the extracted aqueous solution was transferred to a tube, followed by the addition of 0.6 mL of 10% (v/v) sulfuric acid. The total volume was adjusted to 10 mL with NaCl solution, and the mixture was refrigerated at 4°C for 15 minutes. For derivatization, 1 mL of 0.1 M potassium bromate and 1.5 g of potassium bromide were added to the precooled solution. The tube was vortexed, and the reaction was allowed to proceed for 30 minutes at 4°C. The reaction was then quenched by adding 0.1 mL of 0.1 M Na₂S₂O₃ solution. A 4 mL aliquot of the analyte solution was extracted thrice with 4 mL of redistilled ethyl acetate using a separatory funnel. The combined extracts were evaporated to dryness in a rotary vacuum evaporator. The dried residue was reconstituted in 4 mL of *n*-hexane and ultrasonicated for 5 minutes. The *n*-hexane extract was then filtered through cotton using a Pasteur pipette over anhydrous sodium sulfate to remove residual moisture. The purified extract was collected in a GC vial for analysis by GC-ECD (Zhang *et al.*, 2006; Yamazaki *et al.*, 2012; Notardonato *et al.*, 2013; Skinner

et al., 2021). The chromatograms of some samples are shown in Figure 3.

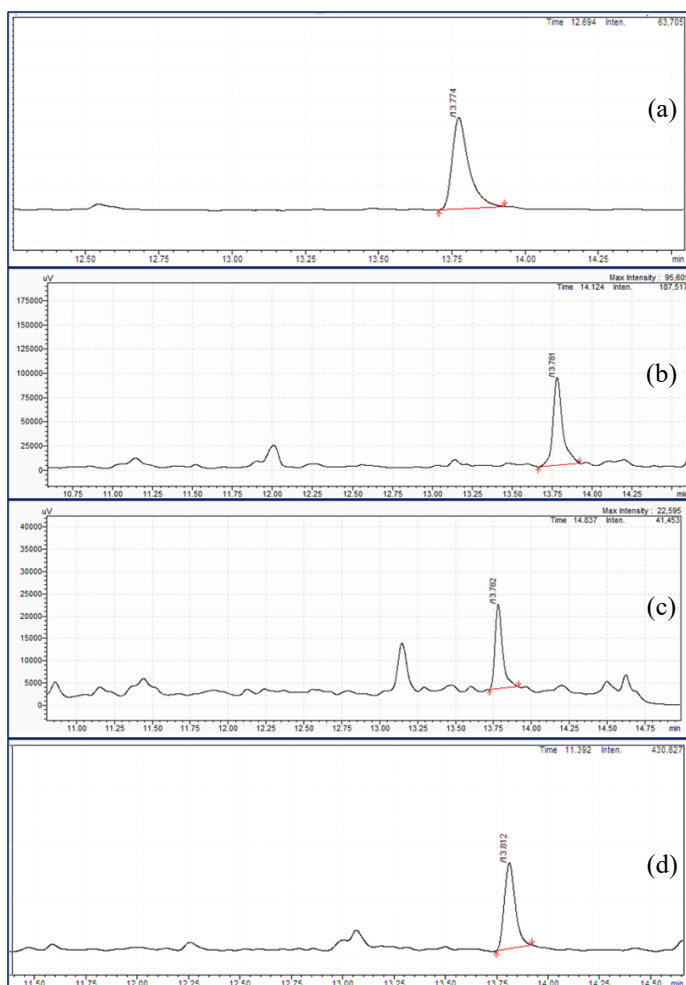


Figure 3. Chromatogram of sample baked products (a) B-6 (b) C-2 (c) P-4(d) BB-1.

2.6 GC-ECD analytical condition

For the quantification of acrylamide, 1 μL of the final test solution was injected onto a gas chromatograph (GC-2030, Shimadzu) equipped with a ^{63}Ni Electron Capture Detector (ECD). Separation was performed using a non-polar HP-5 MS capillary column (30 m \times 250 μm i.d. \times 0.25 μm film thickness, Agilent, USA). Nitrogen served as both the carrier and makeup gas. The temperature program was as follows: an initial temperature of 120 $^{\circ}\text{C}$ (held for 1 min), increased at 12 $^{\circ}\text{C min}^{-1}$ to 140 $^{\circ}\text{C}$ (held for 5 min), followed by an increment of 20 $^{\circ}\text{C min}^{-1}$ to a final temperature of 240 $^{\circ}\text{C}$ (held for 2 min). The injector and detector interface temperatures were maintained at 250 $^{\circ}\text{C}$. A splitless injection mode was used to enhance sensitivity. Identification of acrylamide residues in the samples was achieved by comparing retention times of sample peaks with those of acrylamide standards under identical GC conditions.

2.7 Dietary exposure and risk assessment

In this study, the dietary exposure to acrylamide from bakery products was estimated using a standardized

equation (1). The calculation was based on the consumption patterns of traditional foods, the concentration of acrylamide in these foods, and the average body weight of the population. The formula used for estimating daily acrylamide exposure is as follows:

$$\text{DAE} = \frac{B \times S}{M} \quad (1)$$

Where, DAE = Daily acrylamide exposure (μgkg^{-1} body weight per day), B = Amount of bakery food consumed (g or mL per day; 100 gday^{-1} as a standard consumption value), S = Concentration of acrylamide in bakery foods (μgkg^{-1}), M = Body weight, assumed to be 70 kg for an average adult (Esposito et al., 2020; Aghvami et al., 2023; Başaran et al., 2023; Simões de Borba et al., 2023).

To evaluate the potential health risks associated with dietary acrylamide exposure, a risk characterization was conducted using the Margin of Exposure (MOE) approach. The MOE is a tool used to assess the level of concern for both neurotoxic and carcinogenic effects of acrylamide. The calculations were based on established toxicological benchmarks and the estimated dietary exposure levels.

The Margin of Exposure for neurotoxicity (MOE_n) was calculated for neurotoxic risk assessment as the ratio between the No Observed Adverse Effect Level (NOAEL) for neurotoxic effects and the estimated dietary exposure to acrylamide. The NOAEL for neurotoxicity was set at 0.2 mgkg^{-1} body weight per day, based on toxicological studies (Başaran et al., 2023). The equation (2) is used for MOE_n is as follows:

$$\text{MOE}_n = \frac{\text{NOAEL}}{\text{DAE}} \quad (2)$$

The Margin of Exposure for carcinogenicity (MOE_c) was calculated for carcinogenic risk assessment as the ratio between the Benchmark Dose Lower Confidence Limit (BMDL_{10}) and the estimated dietary exposure. The BMDL_{10} values used were 0.31 mg/kg body weight per day (310 $\mu\text{g/kg}$ body weight per day), representing the dose associated with a 10% increased risk of carcinogenic effects (Basaran and Faiz, 2022). The equation (3) for MOE_c is:

$$\text{MOE}_c = \frac{\text{BMDL}_{10}}{\text{DAE}} \quad (3)$$

A higher MOE_n value indicates a lower risk of neurotoxic effects. An MOE_n value greater than 100 is generally considered to indicate a low level of concern for neurotoxicity. Similarly, a higher MOE_c value suggests a lower risk of carcinogenic effects. A MOE_c value greater than 10,000 is typically considered to indicate a low level of concern for carcinogenicity.

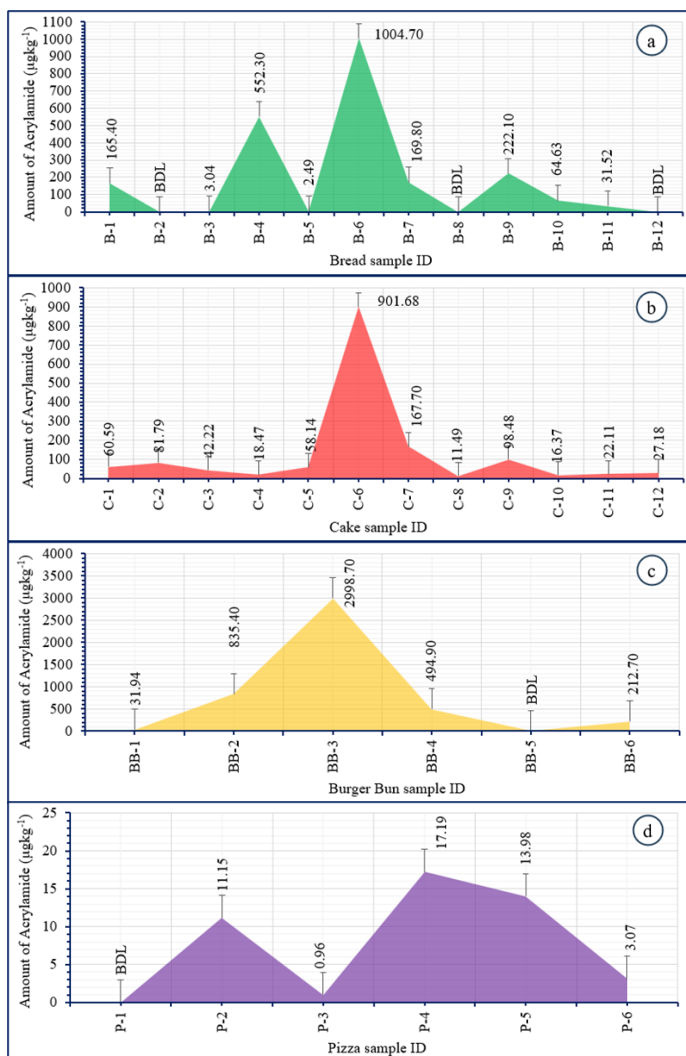


Figure 4. Amount of acrylamide content in different Bakery products (a-d) in Bangladesh.

3. Results and discussion

3.1 Method validation

The validation of the analytical method for acrylamide determination was successfully conducted using calibration solutions in the concentration range of 10 to 200 µg/L. The calibration curve demonstrated satisfactory linearity, with a correlation coefficient (R^2) of 0.9815, indicating a strong relationship between the concentration of acrylamide and the corresponding analytical response (Van Loco *et al.*, 2002). The sensitivity of the method was evaluated by LOD and LOQ, which were found to be 0.54 µg/L and 0.77 µg/L, respectively. These values confirm the method's capability to detect and quantify acrylamide at low concentrations, making it suitable for the analysis of trace levels in complex matrices such as bakery products (Shrivastava and Gupta, 2011; Khaksari *et al.*, 2017). To assess the accuracy and reliability of the method, recovery studies were performed by spiking various bakery samples with a known concentration of acrylamide (33.33 µg/L). The recovery percentages ranged from 62% to 83%, with an average recovery of 73%. While these values indicate acceptable accuracy, the variability in recovery rates across different samples

suggests potential matrix effects or interferences that may influence the analytical performance. Overall, the method demonstrated satisfactory performance in terms of linearity, sensitivity, and accuracy.

3.2 Acrylamide in real samples

The analysis of acrylamide contamination in various bakery products, including bread, cake, burger buns, and pizza, revealed significant findings regarding the prevalence and levels of acrylamide in these food items. A total of 36 bakery samples were analyzed, distributed as follows: 12 bread samples, 12 cake samples, 6 burger bun samples, and 6 pizza samples. The Figure 4(a) presents the levels of acrylamide, a potentially harmful chemical, detected in bread samples collected from 12 different districts in Bangladesh. The acrylamide concentrations in the bread samples are reported in µgkg⁻¹, alongside the benchmark level of 50 µgkg⁻¹ set by the European Union and supported by recent studies (Scientific Opinion on acrylamide in food, 2015; Pogurschi *et al.*, 2021; Sarion *et al.*, 2021) for white bread. The results reveal significant variability in acrylamide levels across the samples. For instance, sample B-6 from Rajshahi recorded the highest concentration (1004.70 µgkg⁻¹), exceeding the EU benchmark by more than 20 times, while samples B-2 (Narayanganj), B-8 (Noakhali), and B-12 (Bhola) showed acrylamide levels below the detection limit (BDL). Other samples, such as B-4 (Mymensingh) and B-7 (Jessore), also exhibited elevated levels (552.30 µgkg⁻¹ and 169.80 µgkg⁻¹, respectively), far surpassing the recommended limit. In contrast, samples like B-3 (Faridpur), B-5 (Sylhet), and B-11 (Bandarban) had relatively low concentrations (3.04, 2.49, and 31.52 µgkg⁻¹, respectively), falling well below the benchmark. A study reported that the acrylamide concentration in the bread samples ranged from 20 to 200 µg kg⁻¹ (Sáez-Hernández *et al.*, 2022). A recent study conducted in Iran revealed that approximately 96% of Sangak bread samples contained acrylamide. Among these, 64.3% of semi-industrial and 33.3% of traditional Sangak bread samples exceeded the benchmark level (Eslamizad *et al.*, 2020). Another study conducted in Nigeria reported that the mean acrylamide concentration in the analyzed bread samples was 163.32 µgkg⁻¹ (Akagha *et al.*, 2022).

The acrylamide levels in cake samples collected from 12 sampling stations across Bangladesh were analyzed and compared with the benchmark level of 66 µgkg⁻¹ set by the European Food Safety Authority (Scientific Opinion on acrylamide in food, 2015) and referenced by other study (Ahmad *et al.*, 2022). The results revealed significant variability in acrylamide concentrations as shown in Figure 4(b), ranging from 11.49 µgkg⁻¹ in Joypurhaat (C-8) to 901.68 µgkg⁻¹

in Khulna (C-6). Notably, six out of the twelve samples (C-2, C-6, C-7, C-9, C-10, and C-12) exceeded the benchmark level, with Khulna (C-6) showing an exceptionally high concentration, approximately 13.7 times higher than the recommended limit. The elevated acrylamide levels in certain samples, such as those from Khulna (C-6) and Tejgaon (C-7), could be attributed to variations in baking conditions, such as higher temperatures or prolonged baking times, which are known to promote acrylamide formation (Scientific Opinion on acrylamide in food, 2015). Conversely, samples like Joypurhaat (C-8) and Savar (C-4) exhibited lower levels, possibly due to better-controlled processing conditions or the use of mitigation strategies, such as asparaginase treatment or reduced sugar content. A recent study revealed that the acrylamide levels in cinnamon cake samples varied between 169.38 and 212.28 μgkg^{-1} (Aghvami *et al.*, 2023). Another study conducted in Nigeria reported that the mean acrylamide concentration in the analyzed cake samples was 305.20 μgkg^{-1} (Akagha *et al.*, 2022). A recent study revealed that, among various bakery products, cake contained detectable levels of acrylamide, with a measured concentration of 71.21 μgkg^{-1} (Ahmad *et al.*, 2022).

The acrylamide levels in burger bun samples from six locations in Dhaka, Bangladesh, were analyzed and compared with the benchmark level of 50 μgkg^{-1} set by the European Union and referenced by other studies (Pogurschi *et al.*, 2021; Sarion *et al.*, 2021). The results revealed significant variability as shown in Figure 4(c), with concentrations ranging from BDL in Mirpur-10 (BB-5) to 2998.70 μgkg^{-1} in Kazipara (BB-3). Notably, four out of six samples (BB-2, BB-3, BB-4, and BB-6) exceeded the benchmark level, with Kazipara (BB-3) showing an exceptionally high concentration, approximately 60 times the recommended limit. The elevated acrylamide levels in samples like Kazipara (BB-3) and Dhanmondi (BB-2) may result from high-temperature baking or prolonged processing times, which are known to promote acrylamide formation. In contrast, the BDL result in Mirpur-10 (BB-5) suggests better control over baking conditions or the use of mitigation strategies.

The acrylamide levels in pizza samples collected from six locations in Dhaka, Bangladesh, were analyzed and compared with the benchmark level of 24 μgkg^{-1} set by the European Food Safety Authority (Scientific Opinion on acrylamide in food, 2015) and referenced by other study (Ahmad *et al.*, 2022). The results showed in Figure 4(d) that all samples were below the benchmark level, with concentrations ranging from BDL in Dhanmondi (P-1) to 17.19 μgkg^{-1} in Motijheel (P-4). Notably, Kotwali (P-3) recorded the lowest level at 0.96

μgkg^{-1} , while Motijheel (P-4) had the highest, still well within the safe limit. The low acrylamide levels in these samples suggest effective control of baking conditions, such as temperature and time, which are critical factors in acrylamide formation. A recent study revealed that, among various bakery products, pizza contained detectable levels of acrylamide, with a measured concentration of 62.42 μgkg^{-1} (Ahmad *et al.*, 2022). The use of quality ingredients and adherence to food safety guidelines may have contributed to these favorable results. These findings indicate that pizza production in Dhaka generally complies with international safety standards, but continuous monitoring and awareness are essential to maintain these levels and further reduce acrylamide exposure.

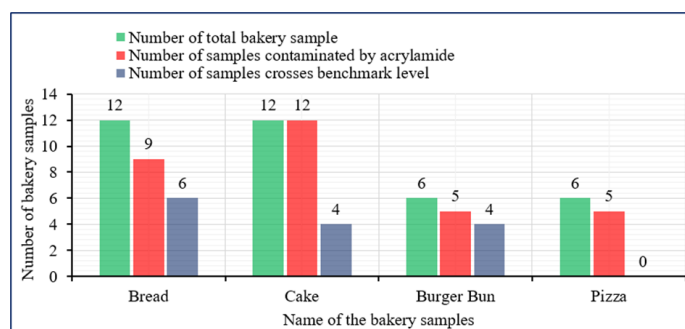


Figure 5. Graphical representation of the analyzed sample contaminated by acrylamide.

The results indicated that acrylamide contamination was widespread across the tested bakery products. Among the 12 bread samples, 9 (75%) were found to be contaminated with acrylamide, while all 12 cake samples (100%) tested positive for acrylamide contamination. Similarly, 5 out of 6 burger bun samples (83%) and 5 out of 6 pizza samples (83%) were contaminated with acrylamide. These findings highlight the pervasive nature of acrylamide in bakery products, which is consistent with previous studies linking acrylamide formation to high-temperature cooking processes such as baking and frying. Furthermore, the study evaluated the extent to which acrylamide levels in these samples exceeded the benchmark levels set by regulatory authorities. Among the contaminated samples, 6 out of 9 bread samples (67%), 4 out of 12 cake samples (33%), and 4 out of 5 burger bun samples (80%) exceeded the benchmark levels. Notably, none of the pizza samples exceeded the benchmark levels, suggesting that the preparation or composition of pizza may result in lower acrylamide formation compared to other bakery products (Figure 5). These findings emphasize the importance of implementing mitigation strategies, such as optimizing baking conditions, using alternative ingredients, or incorporating acrylamide-reducing agents, to minimize acrylamide formation in bakery products. Additionally, regulatory bodies and food manufacturers should collaborate to establish and enforce stricter guidelines to

Table 2. Dietary acrylamide exposure (DAE, μgkg^{-1} body weight per day) of different bakery products of Bangladesh.

Bread	DAE	Cake	DAE	Burger bun	DAE	Pizza	DAE
B-1	0.236	C-1	0.087	BB-1	0.046	P-1	0.000
B-2	0.000	C-2	0.117	BB-2	1.193	P-2	0.016
B-3	0.004	C-3	0.060	BB-3	4.284	P-3	0.001
B-4	0.789	C-4	0.026	BB-4	0.707	P-4	0.025
B-5	0.004	C-5	0.083	BB-5	0.000	P-5	0.020
B-6	1.435	C-6	1.288	BB-6	0.304	P-6	0.004
B-7	0.243	C-7	0.239				
B-8	0.000	C-8	0.016				
B-9	0.317	C-9	0.141				
B-10	0.092	C-10	0.023				
B-11	0.045	C-11	0.032				
B-12	0.000	C-12	0.039				

ensure consumer safety and reduce dietary exposure to acrylamide. Further research is warranted to explore the factors contributing to acrylamide formation in specific bakery products and to develop effective intervention strategies.

3.3 Risk assessment

The dietary acrylamide exposure (DAE) among different bakery products in Bangladesh varied significantly (Table 2). Burger buns exhibited the highest DAE, with BB-3 ($4.284 \mu\text{gkg}^{-1}$ body weight per day) at Kazipara and BB-2 ($1.193 \mu\text{gkg}^{-1}$ body weight per day) at Dhanmondi showing the greatest exposure levels. Among bread samples, B-6 had the highest DAE ($1.435 \mu\text{gkg}^{-1}$ body weight per day) at Rajshahi, while other samples ranged from 0.000 to $0.789 \mu\text{gkg}^{-1}$ body weight per day. Cake samples demonstrated moderate acrylamide exposure, with the highest value observed in C-6 ($1.288 \mu\text{gkg}^{-1}$ body weight per day) at Khulna, whereas most other cakes had values below $0.25 \mu\text{gkg}^{-1}$ body weight per day. Pizza samples exhibited the lowest acrylamide exposure, with DAE values between 0.000 and $0.025 \mu\text{gkg}^{-1}$ body weight per day, except for P-2 ($0.016 \mu\text{gkg}^{-1}$ body weight per day) at Lalbagh and P-5 ($0.020 \mu\text{gkg}^{-1}$ body weight per day) at Azimpur. These variations in acrylamide exposure may be attributed to differences in ingredients, baking conditions, and cooking temperatures. Notably, products with higher heat processing, such as burger buns and certain breads, contained elevated acrylamide levels. The findings suggest that consumption of specific bakery items may

pose higher dietary acrylamide exposure risks, warranting further investigation into mitigation strategies and potential health concerns.

The Margin of Exposure for neurotoxicity (MOE_n) varied widely among different bakery products in Bangladesh (Table 3). Burger buns exhibited the lowest MOE_n , with BB-3 (47) at Kazipara and BB-2 (167) Dhanmondi, indicating a higher potential neurotoxic risk. In contrast, BB-1 (4347) at Mirpur-1 and BB-6 (658) Mohammadpur showed comparatively safer margins. Bread samples displayed a broad range, with B-6 (139) at Rajshahi having the lowest MOE_n , suggesting a potential concern, whereas B-3 at Faridpur and B-5 at Sylhet (both 50,000) indicated minimal risk. Cake samples generally had moderate MOE_n values, with the lowest in C-6 (155) at Khulna and the highest in C-8 at Joypurhaat (12,500). Pizza exhibited the highest safety margins, with P-3 at Kotwali (200,000) and P-6 at Shyamoli (50,000), while P-4 at Motijheel (8000) and P-5 at Azimpur (10,000) remained within safe limits. Undefined values indicate extremely low exposure, leading to negligible risk. The findings suggest that certain bakery products, especially some burger buns and bread, may pose a higher neurotoxic risk due to acrylamide exposure. These variations emphasize the need for stricter control of processing conditions to minimize acrylamide formation in high-risk bakery products.

The Margin of Exposure for carcinogenicity (MOE_c) varied significantly across different bakery products in

Table 3. Margin of exposure for neurotoxicity (MOE_n) of different bakery products of Bangladesh.

Bread	MOE_n	Cake	MOE_n	Burger bun	MOE_n	Pizza	MOE_n
B-1	847	C-1	2298	BB-1	4347	P-1	Undefined
B-2	Undefined	C-2	1709	BB-2	167	P-2	12500
B-3	50000	C-3	3333	BB-3	47	P-3	200000
B-4	253	C-4	7692	BB-4	283	P-4	8000
B-5	50000	C-5	2410	BB-5	Undefined	P-5	10000
B-6	139	C-6	155	BB-6	658	P-6	50000
B-7	823	C-7	837				
B-8	Undefined	C-8	12500				
B-9	631	C-9	1418				
B-10	2173	C-10	8695				
B-11	4444	C-11	6250				
B-12	Undefined	C-12	5128				

Table 4. Margin of exposure for carcinogenicity (MOE_c) of different bakery products of Bangladesh.

Bread	MOE _c	Cake	MOE _c	Burger bun	MOE _c	Pizza	MOE _c
B-1	1313	C-1	3563	BB-1	6739	P-1	Undefined
B-2	Undefined	C-2	2649	BB-2	260	P-2	19375
B-3	77500	C-3	5167	BB-3	72	P-3	310000
B-4	393	C-4	11923	BB-4	738	P-4	12400
B-5	77500	C-5	3735	BB-5	Undefined	P-5	15500
B-6	216	C-6	241	BB-6	1020	P-6	77500
B-7	1275	C-7	1297				
B-8	Undefined	C-8	19375				
B-9	978	C-9	2198				
B-10	3370	C-10	13478				
B-11	6889	C-11	9687				
B-12	Undefined	C-12	7948				

Bangladesh (Table 4). Burger buns showed the lowest MOE_c values, with BB-3 at Kazipara (72) and BB-2 at Dhanmondi (260), indicating a higher carcinogenic risk. In contrast, BB-1 at Mirpur (6739) and BB-6 at Mohammadpur (1020) had relatively higher margins, suggesting lower concern. Bread samples displayed a broad range, with B-6 at Rajshahi (216) and B-4 at Mymensingh (393) at the lower end, while B-3 at Faridpur and B-5 at Sylhet (both 77,500) indicated minimal risk. Cakes exhibited moderate MOE_c values, with C-6 at Khulna (241) having the lowest and C-4 at Savar (11,923) among the highest. Pizza samples had the highest safety margins, with P-3 at Kotwali (310,000) and P-6 at Shyamoli (77,500), while P-4 at Motijheel (12,400) and P-5 at Azimpur (15,500) were within acceptable limits. Undefined values indicate extremely low exposure, leading to negligible risk. These results highlight that certain bakery products, particularly some burger buns and bread, may pose a higher carcinogenic risk due to acrylamide exposure. Controlling acrylamide formation through optimized baking conditions is crucial to reducing potential health risks associated with these products.

Conclusion

This study provides critical insights into acrylamide contamination in bakery products consumed in Bangladesh. The high prevalence of acrylamide, particularly in burger buns, bread, and cakes, raises significant public health concerns. Risk assessment using the Margin of Exposure (MOE) approach indicated potential neurotoxic and carcinogenic risks, with burger buns posing the highest dietary exposure. These findings underscore the urgent need for improved processing techniques to minimize acrylamide formation, such as optimizing baking temperature and time, modifying ingredient composition, and implementing regulatory controls. Regional variations in contamination levels highlight the necessity of continuous monitoring and stricter food safety regulations to protect consumers. Public awareness campaigns on acrylamide risks and safer food preparation methods can further help reduce

exposure. Given the widespread consumption of bakery products in Bangladesh, regulatory agencies should establish and enforce benchmark levels for acrylamide. Future research should focus on developing mitigation strategies and alternative processing methods to ensure safer food production. By addressing these concerns, policymakers, food manufacturers, and consumers can work together to mitigate acrylamide exposure and safeguard public health.

Conflict of interest

The authors declare no conflict of interest.

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