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Hidden risks on the plate: Dietary acrylamide exposure in students using university cafeteria

Elmas Kubra Kucuksullu [a,1], Gizem Aytekin-Sahin [a,b,*,2], Dilsad Ozturk [c,3]

[a] Nuh Naci Yazgan University, Faculty of Health Sciences, Department of Nutrition and Dietetics, Kayseri, Türkiye
[b] Oslo Metropolitan University, Faculty of Health Sciences, Department of Nursing and Health Promotion, Oslo, Norway
[c] Erciyes University Faculty of Engineering, Department of Environmental Engineering, Kayseri, Türkiye

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ABSTRACT

This study aimed to determine the acrylamide level in meals served to university students and to estimate their dietary acrylamide exposure using the duplicate diet method. 125 samples were collected from all meals served at lunch and dinner in the cafeteria for two weeks. Due to the semi-selective menu options, menus were categorized into two groups: "Menu-1" (soup, main dish, side dish, and third course) and "Menu-2" (soup, alternative dish, side dish, and third course). The acrylamide content of the samples was analyzed using high-pressure liquid chromatography (HPLC) with a device equipped with a diode array detector (DAD). Samples found to have acrylamide concentrations below the LOQ (<17.8 μg/kg) were replaced with zero and LOQ values in the lower bound (LB) and upper bound (UB) scenarios, respectively. The margin of exposure (MOE) was used for long-term exposure estimation. A MOE value < 125 indicated a potential concern for human health due to neurotoxic effects, while a MOE value < 10,000 indicated a potential concern for carcinogenic effects. Total acrylamide exposure from Menu-1 and Menu-2 in the LB scenario was calculated as 0.6–0.7 μg/kg/day for males and 0.7–0.8 μg/kg/day for females, respectively. Total acrylamide exposure from Menu-1 and Menu-2 in the UB scenario was 1.1–1.1 μg/kg/day for males and 1.3–1.4 μg/kg/day for females, respectively. In conclusion, the current study showed that acrylamide exposure may be an important concern for food services. It can be minimized by training food service staff in food safety and choosing the correct preparation and cooking methods.

1. Introduction

The heating of food causes chemical reactions that result in the formation of potentially toxic substances known as heat treatment contaminants (Tareke et al., 2002). Among these contaminants, acrylamide has attracted great scientific interest recently due to its presence in various cooked foods. It occurs naturally during cooking processes such as baking, roasting, frying, and industrial processing, particularly in starchy foods, at temperatures above 120 °C and low humidity (EFSA, 2015).

Acrylamide is produced through the Maillard reaction between reducing sugars such as glucose and fructose and asparagine, an amino acid (Xing and Yaylayan, 2021). In 1994, the International Agency for Research on Cancer (IARC) classified acrylamide as a Group 2 A (Possible Carcinogens for Humans) (IARC, 1994). Afterward, Stockholm University and the Swedish National Food Administration first detected acrylamide in foods in 2002, attracting global attention due to its potential health risks (Tareke et al., 2002).

Acrylamide and its metabolite glycidamide can cause various health problems due to its high toxicity, including neurotoxicity, genotoxicity, hepatotoxicity, carcinogenicity, cardiovascular toxicity, and developmental and reproductive system toxicity (IARC, 1994). The acrylamide content in processed foods is closely related to the food preparation method, and the amount in foods varies depending on the type of food and preparation method. Foods with the highest acrylamide content include French fries, chips, corn chips, breakfast cereals, crackers,

* Corresponding author at: Nuh Naci Yazgan University, Faculty of Health Sciences, Department of Nutrition and Dietetics, Kayseri, Türkiye.
E-mail addresses: kucuksullukubra@gmail.com (E.K. Kucuksullu), gasahin@nny.edu.tr (G. Aytekin-Sahin), dilsadapaydin@erciyes.edu.tr (D. Ozturk).
[1] 0009-0009-0978-4183
[2] 0000-0002-6620-9259
[3] 0000-0001-9118-1633

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coffee, bread, biscuits, and other bakery products (FAO & WHO, 2005).

The literature includes various methodologies for assessing dietary acrylamide exposure, incorporating diverse calculation approaches such as deterministic and probabilistic models, consumption estimation techniques (such as 24-hour recall, food frequency questionnaires, or food dietary records), and acrylamide reference levels in foods. These approaches have employed acrylamide reference levels in foods to evaluate exposure patterns, addressing both the general population and specific demographic or risk groups (Claeys et al., 2010; González-Mulero et al., 2023a; Nematollahi et al., 2020). However, most of these studies rely on average values from food consumption records and databases to determine the presence of acrylamide in processed food groups without accounting for differences in cooking methods. The existing limitations hinder reliable results and restrict organizations' ability to monitor acrylamide exposure and advance in this area effectively (Thompson and Subar, 2017).

The European Food Safety Authority (EFSA) recommends duplicate diet studies to improve exposure assessments, provide more accurate data, determine contaminants' dietary intake, and evaluate food safety risks and potential adverse effects on health (EFSA, 2015). In duplicate diet studies, all foods participants consume over a while are collected and analyzed to estimate potential dietary exposure. This method is primarily used to measure exposure to toxic substances in food. However, the number of studies using the duplicate diet method to assess actual dietary acrylamide exposure is limited (González-Mulero et al., 2023a; González-Mulero et al., 2023b; Kawahara et al., 2019; Yamamoto et al., 2018).

During university years, students often move away from parental control and may adopt unhealthy eating habits. Due to busy schedules, stress, and limited access to healthy food options, individuals may consume processed and fast foods, which generally contain higher levels of acrylamide (Escoto et al., 2012). Additionally, in some food service institutions, students are more likely to consume high acrylamide-containing foods due to the preparation of fried foods, lack of necessary controls during food preparation, and prolonged use of frying oil (Mesias and Morales, 2016). However, studies evaluating acrylamide exposure in this group are limited in the literature (Gao et al., 2022; González-Mulero et al., 2023b; Matthys et al., 2005), and most are based on methods that use food consumption estimates with reference levels of acrylamide in foods (Gao et al., 2022; Wyka et al., 2015).

Therefore, the current study aimed to determine the acrylamide level of lunch and dinner meals served to students in a university cafeteria over two weeks and to evaluate the dietary acrylamide exposure of students using the EFSA-recommended duplicate diet method. In addition, this study aimed to contribute to the literature on the acrylamide contents of some Turkish cuisine dishes.

2. Materials and methods

2.1. Chemicals and reagents

Acrylamide standard (C_3H_5NO, 99 %) and acetonitrile (C_2H_3N, 99 %) were obtained from Merck (Darmstadt, Germany). N-hexane (C_6H_{14}, 98 %) was purchased from Kimyalab (Istanbul, Türkiye). Potassium hexacyanoferrate 15 % ($K_3[Fe(CN)_6]$) (Carrez I, 98 %) and zinc sulfate 30 % ($ZnSO_4$) (Carrez II, >99 %) were obtained from Chembio (Istanbul, Türkiye). In preparing all solutions, deionized water was purified using a Milli-Q Integral 5 water purification system (Millipore, Billerica, MA, USA). Cellulose syringe filter units (0.45 μm) were obtained from Isolab (Istanbul, Türkiye). All chemicals, reagents, and solvents were of analytical grade.

2.2. Study design and sample collection

This study was conducted in a university student cafeteria over 14 days from May 13 to May 26, 2024.

The student cafeteria provides two daily meals, lunch and dinner. Both lunch and dinner included a five-course menu: a soup, a main dish, an alternative dish (usually vegetarian), a side dish, and a third course (such as dessert, beverage, fruit, yogurt, or salad). Students can choose between a main course and an alternative dish. Since the cafeteria offers two different options, the menus are categorized as "Menu-1" (soup, main dish, side dish, and third course) and "Menu-2". (soup, alternative dish, side dish, and third course) (Supplementary Material S1). Acrylamide content and its contribution to dietary acrylamide exposure were determined for both menus.

During the fourteen days, 125 lunch samples, 63 dinner samples, and bread samples were collected in the cafeteria. The samples were placed in airtight vacuum bags and labeled. For portion sizes, information was obtained from the food service manager about the standardized weight of one portion of each dish. After collecting, the food samples were photographed and processed in the laboratory. Each meal was allocated FoodEx 2 classifications according to EFSA (EFSA, 2015), a standardized food classification system for data collection and analysis. Samples were prepared according to the procedure recommended by Oroian et al. (2015). According to the procedure, the food collected after each dish was separated, weighed, and homogenized using with a blender. It was stored then at −20 °C until extraction.

2.2.1. Acrylamide extraction

Three grams of the ground sample were placed in a 50 mL centrifuge tube. Next, 20 mL of deionized water and 15 mL of n-hexane were added to extract fat. The centrifuge tubes were shaken for 30 min at 250 rpm using an orbital shaker (Heidolph, Unimax 1010 orbital shaker, Germany) and subsequently centrifuged (Nüve, NF 1200, Turkey) at 3000 rpm for 20 min. The fat layer was removed, and 3 mL of Carez I and 3 mL of Carez II were added to remove proteins and purify the water layer. The tubes were shaken again at 250 rpm for 30 min and centrifuged at 3000 rpm for 20 min. After centrifugation, centrifuge tubes contained three layers: the upper layer (composed of proteins), the intermediate layer (the clear layer containing acrylamide) and the bottom layer (the sample sediment). The middle intermediate was further purified by adding another 3 mL Carrez I and 3 mL Carrez II, then shaken at 250 rpm for 30 min and centrifuged at 3000 rpm for 20 min. The clear layer was finally filtered using a 0.45 μm syringe filter and analyzed.

2.2.2. Acrylamide analysis

Acrylamide analysis was carried out at Erciyes University Technology Research and Application Centre (TAUM) using high-pressure liquid chromatography (HPLC) with a device equipped with a diode array detector (DAD) according to the procedure proposed by Oroian et al. (2015). Samples were analyzed in duplicate, and the results were given in μg/kg.

The pre-cleaning step is analogous to homogenizing (Sebastià et al., 2023). Additionally, it was determined that there would be no interference problem since the sample was homogenized at low rpm without heat. HPLC can separate low acrylamide concentrations, especially when paired with sensitive detection systems such as DAD (Diode Array Detector) or other advanced detectors (Sapyen et al., 2025). Sample extracts were analyzed using an Agilent 1260 Infinity HPLC system (Agilent, California, USA) equipped with liquid chromatography, autosampler, column oven, and 1260 DAD VL. Analysis conditions were determined as 190–240 nm wavelength, water/acetonitrile (90:10, v/v) mobile phase type, 10 μl injection volume, and 1 mL/min flow rate. Six different types of mobile phase were applied to achieve the maximum response of the detector. The six mobile phases were: (1) water/-methanol (90:10, v/v); (2) methanol 10 %: acetonitrile 10 % (50:50, v/v); (3) water/acetonitrile (90:10, v/v); (4) water/formic acid (99.9: 0.1, v/v); (5) water/formic acid (90:10, v/v); and (6) water (100 %). Acrylamide was quantified by measuring the peak area at its retention time and comparing it with a calibration curve constructed using

acrylamide solutions (0.1, 1, 5, 10, 20, 25, 100, and 400 mg/L).

2.2.3. Analytical characteristics

Acrylamide quantification was performed at 200 nm using DAD detection. The mathematical formula of the calibration curve obtained for the acrylamide standard was determined as $y = 92.54x + 74.69$, and the regression coefficient $R^2 = 0.999$ (Supplementary Material S2).

The calibration values were as follows: Precision (%RSD) = 32.29, Residual Precision (%RSD) = 35.71, Bias = 1.55, and %Bias = 0.33 %. The calibration exhibited exceptional accuracy, as demonstrated by the extremely low bias and a coefficient of determination (R^2) of 0.99993, indicating a good fit to the data. With a minimal %Bias of 0.33 %, the calibration effectively minimized systematic error, ensuring high trueness in the measurements. The strong correlation between the predicted and actual values confirmed the robustness and reliability of the calibration model. The calibration demonstrated outstanding performance, with excellent accuracy and a strong linear relationship, ensuring reliable analytical results.

The limit of detection (LOD) was calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) at levels approximating the LOD according to the formula LOD = 3.3 (SD/S). The limit of quantification (LOQ) was computed as LOQ = 10 (SD/S) (ICH, 2005). The regression coefficients were around 0.999, which indicates good performance. According to the formula, LOD was 5.9 μg/kg, and LOQ was 17.8 μg/kg.

The blank matrix chromatograms established no contamination or interference in the current method. The signal was at or below zero, which indicated a clean background. In addition, the 5 mg/L acrylamide sample showed a clear and distinct peak, validating the ability of the method to detect acrylamide at low concentrations. These results supported the reliability of our analytical method (Supplementary Material S2).

2.2.4. Dietary acrylamide exposure estimation

Due to the semi-selective menu options, menus were categorized into two groups: "Menu-1" (soup, main dish, side dish, and third course) and "Menu-2" (soup, alternative dish, side dish, and third course) (Supplementary Material S1). In addition, one packaged bread (50 g) was added to all menus during dietary exposure estimation. Since packaged breads were purchased once a week for the student cafeteria, one bread sample was taken for each week. The acrylamide analysis of the bread samples was performed as described above and included in the dietary exposure estimate.

Foods found to have acrylamide concentrations below the LOQ (<17.8 μg/kg) were replaced with zero and LOQ values in the lower bound (LB) and upper bound (UB) scenarios, respectively, as recommended in the literature for food safety studies. (González-Mulero et al., 2023a; González-Mulero et al., 2021; EFSA, 2018). The acrylamide content of raw foods was assumed to be zero (González-Mulero et al., 2023a; González-Mulero et al., 2021; EFSA, 2018; Tareke et al., 2002). Furthermore, the individual contribution of acrylamide from each meal was determined by multiplying the acrylamide concentration by the weight of the edible portion of the meal (Branciari et al., 2020; EFSA, 2015).

Dietary acrylamide exposure was calculated by summing the acrylamide content of foods consumed at lunch and dinner daily throughout the study period and dividing the total by the average body weight values for females and males (EFSA, 2015). Dietary exposure was calculated using average body weight data (62 kg for females and 77 kg for males) for the Turkish population aged 19–30 (Turkey Nutrition and Health Survey, 2019). The results were expressed as g/kg bw/day.

$$\text{Estimated dietary exposure} = \frac{\text{Food consumption}\left(\frac{\text{kg}}{\text{day}}\right) \text{ x Acrylamide content of food}\left(\frac{\mu\text{g}}{\text{kg}}\right)}{\text{Body weight (kg)}}$$

Risk assessment for acrylamide exposure was conducted by calculating the Margin of Exposure (MOE) for both neurotoxic and carcinogenic effects, as recommended by JECFA and EFSA (FAO & WHO, 2011; EFSA, 2015). The MOE calculation used the BMDL10 dose of 430 μg/kg for neurotoxic effects, previously derived from neurotoxicity studies in rats. A MOE< 125 indicates a potential human health concern for neurotoxic effects. For carcinogenic effects, the $BMDL_{10}$ dose previously calculated in Harderian gland tumors in male mice (170 μg/kg) was used. An MOE< 10,000 for carcinogenic effects indicates a potential risk (EFSA, 2015). The acrylamide exposure risk assessment was performed by calculating the margin of exposure (MOE) for both neurotoxic effects and carcinogenic effects in the LB and UB scenarios according to the following formula:

$$\text{MOE} = \frac{\text{BMDL}_{10}\ (\mu\text{g/kg/day})}{\text{Amount exposed}\ (\mu\text{g/kg/day})}$$

MOE: Margine of Exposure, $BMDL_{10}$: Benchmark Dose Lower 10 %
The study plan was summarized in Fig. 1.

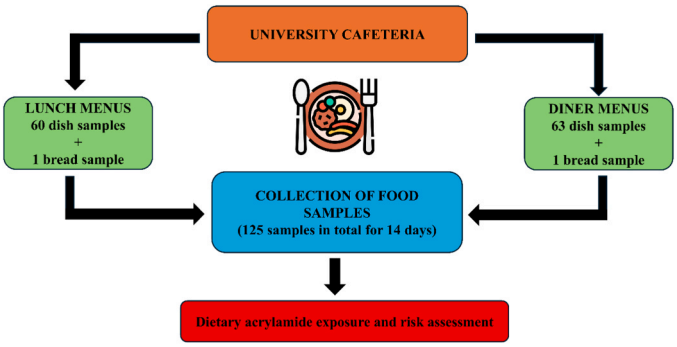


Fig. 1. Study plan.

2.3. Statistical analyses

Statistical Package for Social Sciences software (Version 23.0, USA, IBM Corp., 2015) was used for statistical analysis. Data visualization was performed with GraphPad Prism software (Version 8.0, San Diego, CA, USA). Data were expressed as mean ($\bar{X}$), and standard deviation (SD), and the lowest and highest values were given. The Independent Samples T-test was used for two independent group comparisons. In all analyses, $p < 0.05$ was considered statistically significant.

3. Results and discussion

A total of 125 samples were collected from 60 meals served at lunch, 63 meals served at dinner, and bread from the cafeteria. The acrylamide content of 54 of the 125 food samples collected was found to be above

Table 1
Acrylamide content of meals served in university cafeteria.

Foods	Number (n)	FoodEx 2 Code	Acrylamide content (μg/kg)*	Edible serving (g)	Acrylamide contribution (μg/serving)
SOUPS					
Rice soup with tomato	1	A041Y#F04.A001D	41.6	200	8.3
Chicken soup	1	A041T#F04.A01SP	68.5	200	13.7
Tomato soup	4	A00HQ	25.3 ± 5.6 (20.1–30.2)	200	5.1 ± 1.1 (4.0–6.0)
Cold yogurt soup with rice and chickpeas	1	A02NF	22.3	200	4.5
Turkish dumpling soup	1	A041Y#F28.A07GL	31.8	200	6.4
MAIN DISHES					
Meat dishes					
Kebab with vegetables	1	A03VV# A16GB	66.6	150	10.0
Meatballs					
Turkish fried meat and rice patties	2	A041H#F28.A07GR	44.9 ± 6.6 (40.2–49.6)	100	4.5 ± 0.7 (4.0–5.0)
Grilled meatballs	2	A03XG#F28.A07GZ	25.2 ± 6.4 (20.7–29.7)	100	2.5 ± 0.6 (2.1–2.9)
Turkish fried meat and rice patties with mashed potato	3	A041H#F28.A07GR # A0C6H	67.2 ± 26.3 (40.2–92.8)	150	10.1 ± 4.0 (6.0–13.9)
Chicken dishes					
Baked chicken and potato	1	A01SP#F28.A07GX	282.1	200	56.4
Nugget	3	A03VV	174.6 ± 108.8 (57.5–272.6)	150	26.2 ± 16.3 (8.6–40.9)
Baked chicken with vegetables	1	A03VV#F28.A07GX	20.6	200	3.1
Sesame chicken	1	A015K	308.7	175	54.0
Chicken schnitzel	3	A04DT	80.6 ± 28.1 (48.1–96.8)	150	12.1 ± 4.2 (7.2–14.5)
Vegetable dishes with meat					
Oven-baked vegetable and meat casserole	1	A03VV# A16GB	32.8	200	6.6
Peas with minced meat	1	A03VV# A01BC	28.3	200	5.7
ALTERNATIVE DISHES					
Olive Oil-Based / Meatless Vegetable Dishes					
Cauliflower with béchamel sauce	2	A00FR# A043X	51.8 ± 17.5 (39.5–64.2)	200	10.4 ± 3.5 (7.9–12.8)
Baked cauliflower	1	A00FR	48.4	200	9.7
Peas in olive oil	2	A01BC	33.8 ± 16.3 (22.3–45.3)	200	6.8 ± 3.3 (4.5–9.1)
Spinach in olive oil	1	A00MJ# A043X	38.5	200	7.7
Fried Dishes					
Mixed fried vegetables	1	A03YC	25.3	150	3.8
Fried cauliflower	2	A00FR	71.1 ± 32.9 (47.8–94.3)	150	10.7 ± 4.9 (7.2–14.1)
Fried cauliflower and broccoli	2	A00FR #A00FN	52.8 ± 1.0 (52.1–53.5)	150	7.9 ± 0.2 (7.8–8.0)
French fries	1	A011N	369.6	150	54.4
Others					
Cheese puff pastry	1	A02QE# A00CJ	659.9	100	66.0
SIDE DISHES					
Pastries					
Cheese roll pastry	2	A02QE# A00CJ	275.1 ± 148.2 (170.3–379.9)	150	41.3 ± 22.2 (25.6–57.0)
Cheese puff pastry	1	A02QE# A00CJ	445.7	100	44.6
Pastas					
Roasted noodles	2	A040N	96.0 ± 78.4 (40.5–151.4)	150	14.4 ± 11.8 (6.1–22.7)
Pilafs					
Bulgur pilaf with chickpeas	2	A001D	79.1 ± 37.3	150	11.9 ± 5.6
Vegetable bulgur pilaf	1	A004G# A16GB	39.7	150	6.0
Rice pilaf with orzo	1	A001D# A007J	45.3	150	6.8
THIRD COURSES					
Chocolate cake with chocolate sauce	1	A00BG	63.1	100	6.4
Rice pudding	1	A041E	27.2	200	5.4
Baked potatoes	1	A00ZT#F28.A07GX	206.6	150	31.0
BREAD	2	A004Y	92.0 ± 46.0 (46.0–136.0)	50	4.6 ± 2.3 (2.3–6.8)

Only dishes with an acrylamide concentration higher than LOQ (17.8 g/kg) are detailed (54 from 125 foods/dishes analyzed). The acrylamide content of raw foods was accepted as 0. The acrylamide content of the dishes that served more than once in the menu is given as mean±standard deviation ($\bar{X}$±SD) (Lowest-Highest).

the LOQ (17.8 μg/kg). Dishes with the highest acrylamide content and contributing to acrylamide exposure were cheese puff pastry (659.9 μg/kg, 66 μg/portion and 445.7, 44.6 μg/portion) and French fries (369.6 μg/kg, 37 μg/portion) (Table 1).

The comparison of acrylamide intake from the consumption of meals is given in Table 2. According to the LB scenario, although the two-week average acrylamide intake was 46.2 ± 33.4 μg/day in Menu-1 and 51.5 ± 25.8 μg/day in Menu-2, no statistically significant difference was found ($p = 0.649$). Similarly, according to the UB scenario, the two-week average acrylamide intake was 82.6 ± 25.1 μg/day in Menu-1

Table 2
Comparison of acrylamide intake from the consumption of menus.

	Acrylamide intake (μg/day), LB scenario								
	Monday	**Tuesday**	**Wednesday**	**Thursday**	**Friday**	**Saturday**	**Sunday**	**$\bar{X}$ ± SD**	***p***
Menu−1									
Week 1	13.0	77.7	4.6	72.6	69.5	12.3	62.1		
Week 2	14.9	97.5	54.2	86.8	11.2	59.6	10.7		
2 weeks mean								46.2 ± 33.4	
Menu−2									
Week 1	31.7	90.9	34.4	37.7	71.2	16.3	56.5		0.649
Week 2	13.7	75.1	78.8	52.2	79.7	68.6	13.7		
2 weeks mean								51.5 ± 25.8	
	Acrylamide intake (μg/day), UB scenario								
	Monday	**Tuesday**	**Wednesday**	**Thursday**	**Friday**	**Saturday**	**Sunday**	**$\bar{X}$ ± SD**	***p***
Menu−1									
Week 1	54.3	107.2	63.9	114.2	105.2	78.1	79.8		
Week 2	56.2	115.2	71.9	116.3	52.5	89.1	52.0		
2 weeks mean								82.6 ± 25.1	
Menu−2									
Week 1	67.1	120.4	69.8	61.3	94.8	57.6	80.1		0.812
Week 2	60.9	110.6	102.4	81.7	121.0	98.12	60.9		
2 weeks mean								84.7 ± 22.9	

Menu-1: Soup + main dish + side dish + third course; **Menu-2**: Soup + alternative dish + side dish + third course. Calculations included 50 g of bread. **Abbreviations:** LB, lower bound; UB, upper bound. *Independent Samples T-Test, $p < 0.05$.

and 84.7 ± 22.9 μg/day in Menu-2, and there was no statistically significant difference ($p = 0.812$) (Table 2). The lowest acrylamide intake was observed in week 1, Wednesday, in the LB scenario with Menu-1 selection. This menu included yogurt-based orzo soup (<LOQ μg/kg), meatball rolls (<LOQ μg/kg), rice pilaf with chickpeas and herbs (<LOQ μg/kg), and fruit juice. The highest acrylamide intake occurred in week 2, Friday, in the UB scenario with Menu-2 selection. The highest acrylamide intake occurred in week 2, Friday, in the UB scenario with Menu-2 selection. This menu included tarhana soup (<LOQ μg/kg), cheese puff pastry (659.9 μg/kg), rice pilaf with corn (<LOQ μg/kg) and yogurt (<LOQ μg/kg). In pastries, acrylamide is formed due to the reaction between reducing sugars and asparagine at temperatures above 120 °C. The most important factors affecting acrylamide levels include the presence of free amino acids, cooking time, temperature, and moisture content (Mesias and Morales, 2016). In cheese puff pastry, acrylamide levels may be high due to the amino acid content of the cheese, the presence of reducing sugars in the puff pastry dough, and the dry cooking process at 180 °C. In addition, the oily structure of puff pastry may contribute to acrylamide formation through acrolein production (Rayappa et al., 2021).

The comparison of acrylamide intake by selected menu type is summarized in Fig. 2. Acrylamide intake was 46.2 μg/day in the LB scenario and 82.6 μg/day in the UB scenario for Menu-1 ($p < 0.001$). In Menu-2, acrylamide intake was 51.5 μg/day in the LB scenario and 84.8 μg/day in the UB scenario, and a statistically significant difference was observed between the two scenarios ($p < 0.001$) (Fig. 2). In addition, the acrylamide intake in Menu-2 was higher than in Menu-1. This may be attributed to alternative dishes containing ingredients that contribute to acrylamide formation or involve different cooking methods. Previous studies have demonstrated that acrylamide formation increases, particularly when starchy foods are exposed to high temperatures during cooking (Mogol and Gökmen, 2016; Mesias et al., 2020). Preparing alternative dishes using high-temperature cooking methods such as frying or baking may explain the increased acrylamide levels.

The contribution of meals to acrylamide exposure considering both LB and UB scenarios is given in Fig. 3. In the Menu-1 LB scenario, the contribution of lunch to dietary acrylamide exposure is 37.9 %, and the contribution of dinner to dietary acrylamide exposure is 62.1 %. In Menu-1 UB scenario, the contribution of lunch to dietary acrylamide exposure was 42.2 %, while the contribution of dinner to dietary acrylamide exposure was 57.8 %. In Menu-2, the contribution of lunch to dietary acrylamide exposure was 32.6 % and 67.4 % in the LB

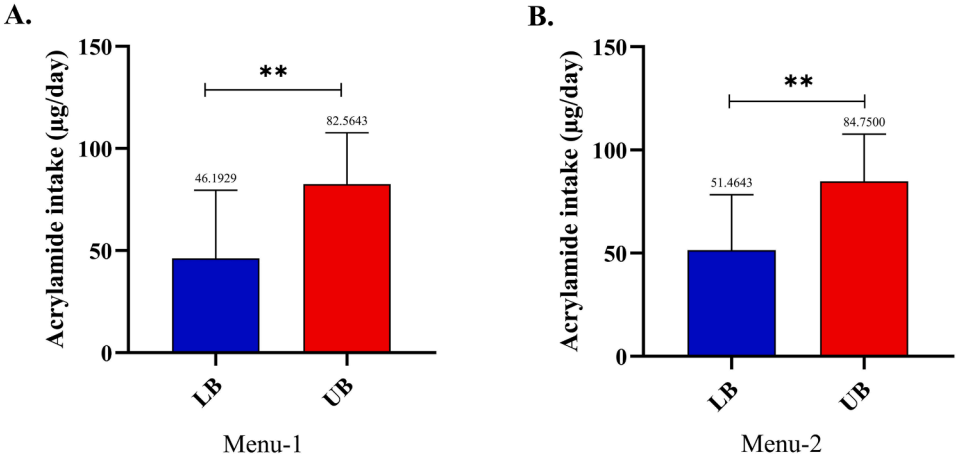


Fig. 2. Comparison of acrylamide intake by selected menu type. **Menu-1:** Soup + Main dish + Side dish + Third course; **Menu-2:** Soup + Alternative dish + Side dish + Third course. Calculations included 50 g of bread. **Abbreviations:** LB, lower bound; ns, not significant; UB, upper bound. **Independent Samples T Test, $p < 0.001$.

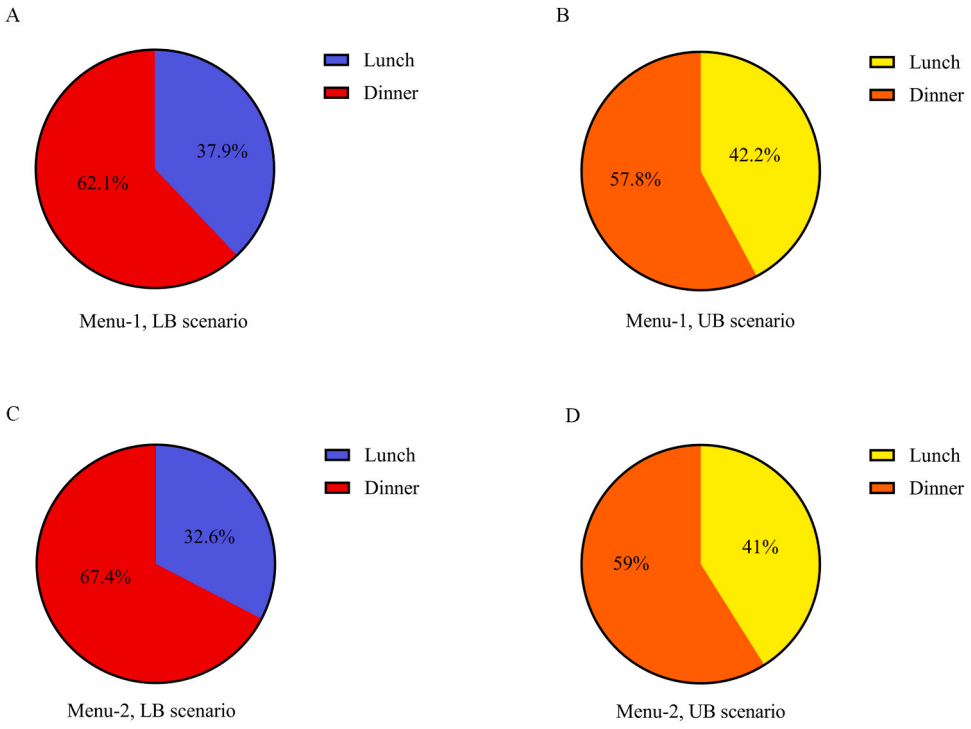


Fig. 3. Contribution of meals to dietary acrylamide exposure. **Menu-1:** Soup + Main dish + Side dish + Third course; **Menu-2:** Soup + Alternative dish + Side dish + Third course. Calculations included 50 g of bread. **Abbreviations:** LB, lower bound; UB, upper bound.

scenario, and the contribution of lunch to dietary acrylamide exposure was 41 % and 59 % in the UB scenario. The reason for the higher contribution of dinner to dietary acrylamide exposure in both scenarios may be that on some days, meals cooked at lunch were reheated and served. In addition, serving cheese puff pastry (659.9 μg/kg), French fries (369.6 μg/kg), cheese roll pastry (275.1±148.2, 183.4 ± 90.3 μg/kg), and nuggets (174.6 ± 108.8 μg/kg) with high acrylamide content in the evening meal increases acrylamide exposure. Potatoes contain high amounts of reducing sugars and asparagine and have higher acrylamide levels than other foods (Mogol and Gökmen, 2016). In previous studies, similar to our study, high acrylamide content was found in French fries (Bakhtiary et al., 2014; Mesías et al., 2019; Ofosu et al., 2019). In coated fried/roasted foods such as nuggets, acrylamide formation occurs mainly on the surface and depends on the composition of the coating. Using coating materials such as milk, egg, salt, potato starch, spices, and breadcrumbs can increase acrylamide formation by reducing sugar and

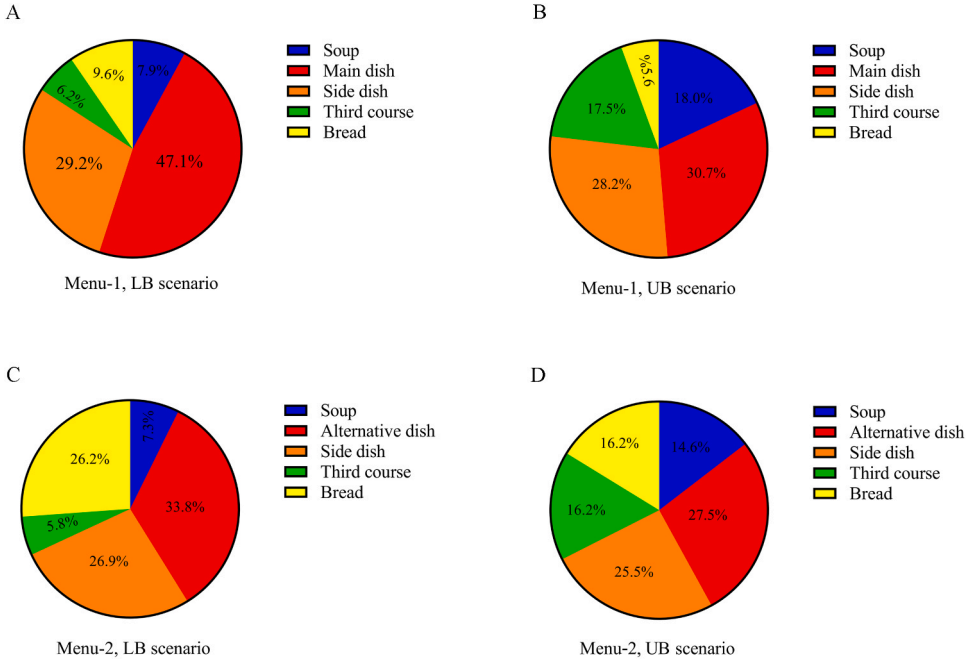


Fig. 4. Contribution of dish types to dietary acrylamide exposure. **Menu-1:** Soup + Main dish + Side dish + Third course; **Menu-2:** Soup + Alternative dish + Side dish + Third course. **Abbreviations:** LB, lower bound; UB, upper bound.

asparagine levels in the food (Perera and Embuscado, 2014).

The contribution of dish types to dietary acrylamide exposure is summarized in Fig. 4. The main dishes (47.1 % - 30.7 %) had the highest contribution to acrylamide exposure in Menu-1 LB and UB scenarios, followed by the side dishes (29.2 % - 28.2 %). The acrylamide content per kg in main dishes was relatively low; however, the acrylamide amount per serving was higher due to their larger portion sizes than other dishes. Similarly, in the study conducted by (González-Mulero et al., 2023b), although main dishes had lower acrylamide content compared to other dishes, they were the food category that contributed the most to acrylamide exposure.

In the Menu-1 LB scenario, the lowest contribution to acrylamide exposure was found to be third courses (6.2 %), while in the UB scenario, it was bread (5.6 %), (Fig. 4). This change emphasizes the importance of including foods and meals with minimal acrylamide levels below the LOQ in the comprehensive assessment of dietary acrylamide intake.

In Menu-2, alternative dishes contributed the highest amount to acrylamide exposure in both the LB (33.8 %) and UB (27.5 %) scenarios, followed by side dishes (26.9 % in LB and 25.5 % in UB) (Fig. 4). The third course had the lowest contribution to acrylamide exposure in the LB scenario (7.3 %), while soup had the lowest contribution in the UB scenario (14.6 %) (Fig. 4). Alternative meals contributed the most to acrylamide exposure because they included dishes with high acrylamide content, such as cheese puff pastry (659.9 μg/kg) and French fries (369.6 μg/kg). In cheese puff pastry, the amino acid content of the cheese, the amount of reducing sugar in the dough, and the high-temperature cooking of the pastry may have resulted in high acrylamide content, as explained above. When we examined French fries, the acrylamide content was similar to the findings of a study conducted in school canteens in Spain (329 μg/kg) (Mesias et al., 2020). However, it was found below the reference value (500 μg/kg) set by the EU (European Commission, 2017). Potatoes have higher acrylamide levels than other foods because they contain high amounts of reducing sugars and asparagine (Mogol and Gökmen, 2016). At the same time, various factors, such as cooking method, temperature, time, pH, surface area, moisture content, and growing and storage conditions, also affect acrylamide formation (Govindaraju et al., 2024). Therefore, the study results may differ.

This study also assessed dietary acrylamide exposure based on the students' gender. In most of the studies in the literature, dietary exposure assessments are based on questionnaires such as food consumption frequency questionnaires and 24-hour dietary recalls. However, these questionnaires cannot track variables such as ingredients used and cooking techniques that may vary by each individual and affect acrylamide exposure (Claeys et al., 2010; González-Mulero et al., 2023b; Nematollahi et al., 2020). To address these limitations, companion dietary studies that assess both the amount of food consumed and the concentration of the chemical by a specific group provide a more realistic estimate of the risk associated with exposure (González-Mulero et al., 2023b; Yamamoto et al., 2018). Therefore, this study used the duplicate diet method to evaluate dietary acrylamide exposure. Total acrylamide exposure from Menu-1 and Menu-2 in the LB scenario was calculated as 0.6–0.7 μg/kg/day for males and 0.7–0.8 μg/kg/day for females, respectively (Table 3). Total acrylamide exposure from Menu-1 and Menu-2 in the UB scenario was 1.1–1.1 μg/kg/day for males and 1.3–1.4 μg/kg/day for females, respectively. The higher exposure observed in females may be attributed to their lower body weight.

In a previous study by Gao et al. (2022), acrylamide exposure was determined to be 0.48 μg/kg/day for males and 0.53 μg/kg/day for females. In another study conducted in Peru, where acrylamide exposure was calculated using a food consumption frequency questionnaire, the exposure levels were reported as 1.37 μg/kg/day for male students and 1.41 μg/kg/day for female students (Tacca Ortiz, 2020). Nonetheless, variations in the methodologies employed to evaluate acrylamide exposure complicate comparisons with studies in the literature. In addition, it has been reported that the estimate of acrylamide exposure using consumption estimates may be higher than that of acrylamide exposure using the duplicate diet method (Yamamoto et al., 2018). Moreover, since the studies were conducted in different countries, it is likely that the characteristics of the culinary culture and the dietary habits of the society also affect dietary acrylamide exposure.

Acrylamide and its metabolite glycidamide are genotoxic and carcinogenic substances that can cause DNA damage and lead to cancer. The MOE approach is employed in risk assessment for genotoxic compounds lacking specified tolerable daily intake. A MOE value of less than 125 indicates a potential human health concern for neurotoxic effects, while a MOE value of less than 10,000 indicates a potential risk for carcinogenic effects (EFSA, 2015). In our study, all MOE values calculated for neurotoxic effects were above 125, and it was determined that students' dietary acrylamide exposure did not pose a potential risk for neurotoxicity. However, MOE values calculated for carcinogenic effects were below 10,000 and ranged between 124.4 and 283.4 (Table 3). It was also found that MOE values calculated for carcinogenic effects were lower in females than in males.

These findings suggested that females have a higher risk than males. This may be due to the lower average body weight of females than males. In the study conducted by González-Mulero et al. (2023b) on university students, acrylamide exposure was assessed based solely on lunch consumption. The MOE values for neurotoxic effects ranged from 982 to 5136, while the MOE values for carcinogenic effects ranged from 388 to 2031. Similarly, total dietary acrylamide exposure was determined in university students in China, and MOE values were reported to be below 10,000 (Gao et al., 2022). Similar to our study, previous studies have concluded that dietary acrylamide exposure in university students does not pose a risk in terms of neurotoxic effects; however, its potential carcinogenic effects should not be overlooked.

The current study has some strengths and limitations. One of its strengths is risk assessment using MOE calculations, which can guide public health policies. Second, the EFSA-recommended duplicate diet method was used in the present study to analyze actual portions and acrylamide concentrations for more realistic results. Finally, a detailed risk analysis was conducted using two different exposure scenarios (LB and UB) based on students' choices between main dishes and alternative dishes.

However, this study has some limitations. First, it was limited to one student cafeteria in a university. In addition, the contribution of the two-week menu to students' acrylamide exposure was assessed, which limits

Table 3
Risk assessment of dietary acrylamide exposure in students using the university cafeteria.

	Male				Female			
	LB scenario		UB scenario		LB scenario		UB scenario	
	Menu-1	Menu-2	Menu-1	Menu-2	Menu-1	Menu-2	Menu-1	Menu-2
Dietary acrylamide exposure (μg/kg/day)	0.6	0.7	1.1	1.1	0.7	0.8	1.3	1.4
MOE for neurotoxic effects	716.8	643.3	401.1	390.1	577.2	518.0	322.9	314.6
MOE for carcinogenic effects	**283.4**	**254.3**	**158.5**	**154.5**	**228.2**	**204.8**	**127.7**	**124.4**

Menu-1: Soup + Main dish + Side dish + Third course; **Menu-2:** Soup + Alternative dish + Side dish + Third course. Calculations included 50 g of bread. **Abbreviations:** LB, lower bound; MOE, Margin of Exposure; UB, upper bound.

the generalizability of the results. Second, leftovers were not considered, which may lead to overestimating exposure. Third, the results may appear localized due to the nature of food culture. However, these studies are valuable for understanding the contribution of dishes to acrylamide exposure.

Nevertheless, to our knowledge, this is the first study in which acrylamide exposure was determined in Turkish university students using the duplicate diet method. Additionally, this study is one of the limited sources on the acrylamide content of Turkish dishes. The study is thought to shed light on other comprehensive studies in this field. Future studies should be conducted in multiple centers and investigate longer-term exposure.

4. Conclusions

In conclusion, this study evaluated the acrylamide content of the menus served to students in a university cafeteria for two weeks, and their contribution to dietary acrylamide exposure. In addition, dietary acrylamide exposure estimation and exposure risk assessment were performed based on the consumption of these menus. It was observed that the MOE values calculated for carcinogenic effects were lower than 10,000 and, therefore, may pose a potential risk for carcinogenic effects in university students. Considering these results, it is important to conduct more studies on this issue to create guidelines to raise public awareness and to include this issue in dietary guidelines. Training food service staff in healthy food preparation and cooking and informing students about healthy nutrition and healthy food choices may help reduce acrylamide exposure.

CRediT authorship contribution statement

Kucuksullu Elmas Kubra: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Aytekin-Sahin Gizem:** Writing – review & editing, Visualization, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. **Ozturk Dilsad:** Writing – review & editing, Methodology, Investigation.

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Declaration of Competing 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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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jfca.2025.107576.

Data availability

Data will be made available on request.

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