



# Determining the Levels of Acrylamide in Some Traditional Foods Unique to Turkey and Risk Assessment

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## Abstract

In this study, exposure risk assessment was made by determining the acrylamide levels of some traditional foods frequently consumed by the Turkish society and registered geographical indication. For this purpose, acrylamide levels of 20 traditional foods [7 meat products, 3 loaves of bread, 3 bagels (simit), and 7 desserts] obtained from different bakeries, patisseries, and restaurants were determined by LC-MS/MS. Acrylamide levels were determined between 12.7 - 299  $\mu\text{g}/\text{kg}$  in meat products, 11.8 - 69.3  $\mu\text{g}/\text{kg}$  in bread, 11.8 - 179  $\mu\text{g}/\text{kg}$  in bagels, 11.7 - 85.0  $\mu\text{g}/\text{kg}$  in baked desserts, and 32.3 - 527  $\mu\text{g}/\text{kg}$  in deep-fried desserts. According to the portion size, the food with the highest acrylamide level in meat products is Adana kebab (17.70  $\mu\text{g}/180\text{ g}$ ). Formulation and cooking techniques are thought to be the main determinants of acrylamide level detected in traditional foods. Dietary acrylamide exposure was calculated according to the deterministic model. Exposure was calculated as 0.20, 0.53, and 0.98  $\mu\text{g}/\text{kg}$  bw per day for good, average and bad scenarios, respectively. The calculated acrylamide exposure value is below the reference values stated by FAO/WHO. The acrylamide dietary exposure was not of concern concerning neurotoxicity and carcinogenicity. The results can be used to reduce acrylamide levels in foods and risk assessment studies.

**Keywords:** Acrylamide, Bakery Products, Meat Products, Desserts, Adana Kebab, Baklava, Dietary Exposure

## 1. Background

Acrylamide is a colorless, odorless compound that is easily soluble in water, methanol, and acetone and is a widely used chemical agent in the industry (1). The presence of acrylamide in foods was first announced in Sweden in 2002 (2). Acrylamide was reported to occur at high levels with heat treatment (120°C and above), especially in foods rich in asparagine amino acids and reducing sugars, and the Maillard Reaction played an important role in this formation (3). Besides, some studies show that acrolein, aspartic acid, carnosine, B-alanine, and pyruvic acid are converted into acrylamide by various reactions (4).

Since acrylamide has a low molecular weight (71.08 g/mol), it is easily absorbed by the organism and dispersed in the body. In addition, acrylamide can form compounds with DNA, RNA, and proteins by undergoing various chemical reactions in the organism. The European Food Safety Authority (EFSA) reported that acrylamide has a genotoxic and neurotoxic effect on experimental animals (5). International Agency for Research on Cancer (IARC) defined acrylamide in 1994 in group 2A, which is a probably car-

cinogen for humans (6). Besides, some researchers have stated that the risk of developing various types of cancer increases with dietary acrylamide intake (7-9). In 2011, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) noted that neurotoxic NOAEL levels in mice are 0.2 mg/kg bw per day (10).

The European Commission made recommendations in 2007 that EU member states should monitor foodstuffs with high levels of acrylamide content and dietary acrylamide exposure (11). When the literature is examined, it is possible to find many studies on both acrylamide levels and exposure related to consumption of these foods in French fries, potato chips, bread, biscuits, breakfast cereals, baby food, and coffee (12-21). Studies conducted by the European Commission show that the average acrylamide levels in foods vary between 24 - 2,942  $\mu\text{g}/\text{kg}$  and the average acrylamide exposure is in the range of 0.4 - 1.9  $\mu\text{g}/\text{kg}$  bw per day (5). The European Commission has set the benchmark levels for certain kinds of food. The benchmark levels for the presence of acrylamide in foodstuffs are as follows: (1) French fries (ready-to-eat) 500  $\mu\text{g}/\text{kg}$ ; (2)

potato-based crisps, crackers, and other potato products 750  $\mu\text{g}/\text{kg}$ ; (3) soft bread 50 - 100  $\mu\text{g}/\text{kg}$ ; (4) breakfast cereals 150 - 300  $\mu\text{g}/\text{kg}$ , biscuits and wafers 350  $\mu\text{g}/\text{kg}$ ; (5) crackers (the exception of potato-based) 400  $\mu\text{g}/\text{kg}$ ; (6) crispbread 350  $\mu\text{g}/\text{kg}$ ; (7) gingerbread 800  $\mu\text{g}/\text{kg}$ ; (8) roast coffee 400  $\mu\text{g}/\text{kg}$ ; (9) instant (soluble) coffee 850  $\mu\text{g}/\text{kg}$ ; (10) coffee substitutes 500 - 4,000  $\mu\text{g}/\text{kg}$  baby foods (processed cereal-based foods) 40  $\mu\text{g}/\text{kg}$ ; (11) biscuits and rusks (for infants and young children) 150  $\mu\text{g}/\text{kg}$  (22). When the literature on acrylamide levels in foods is examined, it is understood that the focus is mostly on certain foods that are frequently consumed by all segments of the population. However, it can be said that the importance of traditional foods in daily nutrition is partially neglected.

Yet, the traditional foods, which are frequently consumed by the public and meet a significant part of daily vitamin-mineral and protein needs and should be handed down to next generations, are acknowledged as cultural heritage (23-25). Interest in traditional foods has increased due to the close relationship between nutrition and health concepts (26-28). Many countries develop policies focusing on traditional foods for rural development, sustainability, entrepreneurship, efficient use of resources, and gastronomy tourism (29, 30). Thus, it is thought-provoking that acrylamide studies on traditional foods, which have become increasingly important in recent years and have an important share in our diet, are limited. This loss of data also adversely affects dietary acrylamide exposure studies.

Studies on acrylamide still maintain their importance in the scientific world due to its presence in different levels in many foods in our daily diet and the exposure that occurs with the consumption of these foods, and the potential dangers they pose for human health. Furthermore, the Joint FAO/WHO Expert Committee on Food Additives emphasized that there is little information on the level and formation of acrylamide in foods in developing countries. However, even this information is crucial for developing various strategies to minimize acrylamide concentrations in foods (10, 31). The number of studies on the acrylamide level carried out in Turkey is quite limited, and they have focused mainly on some foods similar to those in the foreign literature. However, Turkish cuisine culture is very rich in traditional foods and is among the world's three most important cuisines (21, 32, 33). This study aims to determine the acrylamide level of some traditional foods specific to Turkish cuisine and assess the risk of acrylamide exposure.

## 2. Methods

### 2.1. Samples

Traditional foods in Turkey are protected by Law no. 6769 Intellectual Property Law by the Turkish Patent and Trademark Office (TPTO), and more than 800 products have been registered so far (34, 35). The traditional foods, which constitute the material of this study, were selected from the products registered by TPTO with a geographical indication or applied for registration and having high public awareness and consumption.

In line with the research purposes, the following foods (1 portion) were purchased from the stated different bakeries, patisseries, and restaurants: (1) Adana kebabı (n = 3); (2) lahmacun (n = 3), (3) künefe (n = 3); (4) baklava (pistachio) (n = 5); (5) kaymaklı ekmek kadayıfı (crumpets in thick syrup) (n = 3); (6) halka (n = 3); (7) lokma (n = 3) and tulumba dessert (n = 3); (8) stuffed meatballs (n = 2); (9) Akhisar meat patty (n = 2); (10) İnegöl meat patty (n = 2) from Erzurum (a province of Turkey); (11) Akçaabat meat patty (n = 3) and Vakfıkebir bread (n = 2) from Trabzon (a province of Turkey); (12) laz böreği (pastry) (n = 2); (13) kavurma (n = 2); (14) baston bread (n = 2) from Rize (a province of Turkey); (15) Gümüşhane bread (n = 2) from Gümüşhane (a province of Turkey); (16) simit (n = 3 from each, respectively) from Rize, Samsun, and Ankara (some provinces of Turkey). The composition of the traditional foods included in this research and the preparation processes for consumption, including cooking techniques, are explained as a footnote in Tables 1 - 3. All of the foods (n = 53) (7 traditional meat products, 6 traditional bakery products, and 7 traditional desserts) were crushed in a mortar (cleaned before each use) to be homogeneous and then analyzed, and acrylamide level of 1 portion of traditional food was calculated.

### 2.2. Stock and Working Solution

Acrylamide (99%) (1 mg/mL) and acrylamide- $\text{d}_3$  (1 mg/mL) (Sigma-Aldrich; St. Louis, MO) stock and working standards were prepared in HPLC-grade water with 0.1% formic acid (ISOLAB; Wertheim, Germany). To prepare working solutions, the stock solution was diluted with acetonitrile:distilled water (75: 25 v/v) with 0.1% formic acid.

### 2.3. Acrylamide Extraction

Ten grams samples from all foods were weighed in a 250 mL glass beaker, and 90 mL water was added. All samples were mixed in a rotating shaker for 30 min. Nine milliliters samples were placed into a 50 mL centrifuge tube. Then 1 mL (100 ng/mL) acrylamide- $\text{d}_3$  and 5 mL n-hexane

**Table 1.** Acrylamide Levels of Traditional Meat Products

Traditional Foods	Registration No.	City	N	Average Acrylamide Level [Min - Max] ( $\mu\text{g}/\text{kg}$ )	Acrylamide Level Corresponding to 1 Portion <sup>a</sup>
Adana kebab <sup>b</sup>	65	Adana	3	98.3 [12.9 - 299]	17.7 $\mu\text{g}/180\text{ g}$
Kavurma <sup>c</sup>	462	Rize	2	31.3 [12.7 - 69.6]	3.10 $\mu\text{g}/100\text{ g}$
Lahmacun <sup>d</sup>	236	Gaziantep	3	72.5 [13.6 - 162]	5.80 $\mu\text{g}/80\text{ g}$
Akcaabat meat patty <sup>e</sup>	132	Trabzon	2	53.1 [37.3 - 82.7]	5.30 $\mu\text{g}/100\text{ g}$
Akhisar meat patty <sup>f</sup>	322	Manisa	2	30.7 [17.0 - 51.2]	3.10 $\mu\text{g}/100\text{ g}$
İnegöl meat patty <sup>g</sup>	78	Bursa	2	47.7 [17.8 - 91.3]	4.80 $\mu\text{g}/100\text{ g}$
Stuffed meatball <sup>h</sup>	551	Adana	2	129 [40.6 - 309]	12.9 $\mu\text{g}/100\text{ g}$

Abbreviation: N, number of samples.

<sup>a</sup> The portion size may vary depending on the restaurant and the consumer.

<sup>b</sup> It is a product made by using traditional production techniques from a mixture of lamb minced meat (used by a big knife which is called "zırh"), tail fat, garlic, onion, chili pepper, black pepper, salt, hot pepper paste, and parsley and cooked on oak charcoal (36).

<sup>c</sup> It is the name of the product made with chopping the meat of bovine or ovine in cubes, cooking with tallow and adding salt, and it is finally frozen. This product is used as a filling material in pita and is cooked in traditional stone ovens (37).

<sup>d</sup> It is a product that is consumed by putting a mixture of ground beef, ground lamb, onion, garlic, parsley, tomato, green pepper, pepper paste, tomato paste, ground pepper, chili pepper, salt, black pepper, and cumin into a thinly rolled yeast dough and cooked in traditional stone ovens (38).

<sup>e</sup> It is a product that is made by shaping a mixture of ground beef, tallow, garlic, bread, and salt, and then cooked on charcoal or deep-fried (39).

<sup>f</sup> It is a product made by shaping a mixture of ground beef, onion and salt, cooked on charcoal or deep-fried (40).

<sup>g</sup> It is a product that is consumed by cooking on charcoal or deep frying, made with a mixture of ground beef, lamb mince, bread or breadcrumbs, onion, salt, and sodium bicarbonate (41).

<sup>h</sup> The mixture consisting of bulgur, semolina, water, wheat flour, tomato paste, pepper paste, sunflower oil/olive oil, salt, and black pepper is thoroughly kneaded, and the inside of the dough is left empty and its traditional shape is given. An internal mixture is prepared from ground beef/lamb, sunflower oil, onion, salt, black pepper, and chili peppers. The mixture is filled into the dough and fried in deep oil (sunflower oil) (42).

**Table 2.** Acrylamide Levels of Traditional Bakery Products

Traditional Foods	Registration No.	City	N	Average Acrylamide Level [Min - Max] ( $\mu\text{g}/\text{kg}$ )	Acrylamide Level Corresponding to 1 Portion <sup>a</sup>
Vakfikebir bread <sup>b</sup>	372	Trabzon	2	36.0 [15.4 - 51.2]	6.50 $\mu\text{g}/180\text{ g}$ <sup>c</sup>
Rize baston bread <sup>d</sup>	439	Rize	2	40.6 [11.8 - 69.3]	7.30 $\mu\text{g}/180\text{ g}$ <sup>c</sup>
Gümüşhane bread <sup>e</sup>	221	Gümüşhane	2	34.2 [17.2 - 56.5]	6.15 $\mu\text{g}/180\text{ g}$ <sup>c</sup>
Ankara simit <sup>f</sup>	235	Ankara	3	106 [32.7 - 179]	6.35 $\mu\text{g}/60\text{ g}$
Rize simit <sup>g</sup>	410	Rize	3	12.5 [11.8 - 13.4]	0.75 $\mu\text{g}/60\text{ g}$
Samsun simit <sup>h</sup>	176	Samsun	3	41.1 [12.7 - 69.6]	2.50 $\mu\text{g}/60\text{ g}$

Abbreviation: N, number of samples.

<sup>a</sup> The portion size may vary depending on the consumer.

<sup>b</sup> It is a product that is produced by kneading the mixture of bread wheat flour, sourdough, water, and salt according to the traditional technique, and then shaping and baking it in stone ovens (43).

<sup>c</sup> Bread consumption is considered to be 180 grams per day per capita (44).

<sup>d</sup> It is a product that is produced by kneading a mixture of bread wheat flour, fresh yeast, water, and salt according to the traditional technique, and then shaping and cooking it in traditional stone ovens (45).

<sup>e</sup> It is a product that is produced by kneading a mixture of bread wheat flour, fresh yeast, sourdough, water, and salt according to the traditional technique, and then shaping and cooking it in traditional stone ovens (46).

<sup>f</sup> It is a product that is made by kneading the mixture consisting of wheat flour, fresh yeast, water, and salt according to the traditional technique. After shaped, firstly, it is dipped in grape molasses and roasted sesame. Finally, it is cooked in ovens (47).

<sup>g</sup> It is a product produced by kneading a mixture of wheat flour, fresh yeast, water, and salt according to the traditional technique, shaping and dipping it into grape molasses and consumed by baking in ovens (48).

<sup>h</sup> It is a product produced by kneading a mixture of wheat flour, fresh yeast, water, and salt according to the traditional technique, shaping and dipping it first in grape / mulberry / apple / pear molasses and then in sesame, and cooked in ovens (49).

(ISOLAB; Wertheim, Germany) were added. The centrifuge tubes were capped and shaken or vortexed for 2 min to mix contents. The tubes were centrifuged at 9000 rpm for 15 min with an Allegra X-30R centrifuge equipped with a C0650 head (Beckman Coulter; Palo Alto, CA). A pipet was used to transfer a 5 mL aliquot of the clarified aque-

ous layer to a filtration tube through a Maxi-Spin 0.45  $\mu\text{m}$  PVDF (ISOLAB; Wertheim, Germany), and this tube was centrifuged at 9000 rpm for 3 min. Oasis HLB cartridges (Waters; Milford, MA) were preconditioned with the first 3.5 mL MeOH (ISOLAB; Wertheim, Germany) and then 3.5 mL water. The solvents used for column conditioning were dis-

**Table 3.** Acrylamide Levels of Traditional Desserts

Traditional Foods	Registration No.	City	N	Average Acrylamide Level [Min - Max] ( $\mu\text{g}/\text{kg}$ )	Acrylamide Level Corresponding to 1 Portion <sup>a</sup>
Künefe <sup>b</sup>	101	Hatay	3	31.3 [11.7 - 69.6]	5.47 $\mu\text{g}/175\text{ g}$
Baklava <sup>c</sup>	95	Gaziantep	5	40.6 [11.8 - 69.3]	6.49 $\mu\text{g}/160\text{ g}$
Laz böreği <sup>d</sup>	407	Artvin	2	11.7 [11.7 - 11.7]	1.17 $\mu\text{g}/100\text{ g}$
Kaymaklı ekmek kadayıfı <sup>e</sup>	473	Afyonkarahisar	3	67.7 [50.3 - 85.0]	10.8 $\mu\text{g}/160\text{ g}$
Halka dessert <sup>f</sup>	555	Adana	3	295 [63.8 - 527]	37.0 $\mu\text{g}/125\text{ g}$
Tulumba dessert <sup>g, h</sup>	-	Adana	3	128 [32.3 - 224]	12.8 $\mu\text{g}/100\text{ g}$
Lokma dessert <sup>i, h</sup>	-	Adana	3	113 [50.3 - 176]	11.3 $\mu\text{g}/100\text{ g}$

Abbreviation: N, number of samples.

<sup>a</sup> The portion size may vary depending on the consumer.

<sup>b</sup> First, butter and syrup are added to the shredded phyllo dough made from wheat flour. Then, unsalted cheese, unique to the region, is placed between shredded phyllo dough, and it is cooked over low heat. It is a type of dessert that is consumed by pouring hot syrup (made with boiling sugar, water, and a small amount of lemon juice) immediately after cooking (50).

<sup>c</sup> The dough is prepared by kneading the wheat flour, butter, egg, water, and salt mixture in accordance with the technique and rolled out to be very thin with a dough roller. Butter, cream, and pistachios are placed between each layer of dough and baked in the oven. Finally, boiling syrup (made with boiling sugar, water, and a small amount of lemon juice) is poured on it (51).

<sup>d</sup> The dough is prepared by kneading the wheat flour, egg, milk, water, and salt mixture in accordance with the technique by using a dough roller. Pudding is prepared with milk, sugar, wheat flour, salt, eggs, yoghurt, black pepper, and butter. Pudding is poured over the doughs, sugar and butter are added to the pudding, and then baked in the oven. It is recommended to serve it cold (52).

<sup>e</sup> It is prepared with bread wheat flour, water, fresh yeast, salt into a dough in accordance with its technique, and soaking the dry shredded phyllo dough, which has a pita-like structure but has dense pore amount, with syrup consisting of white sugar, water, and sour salt components, and then baked in the oven. It is recommended to serve it cold with special Afyon cream (53).

<sup>f</sup> Wheat flour, semolina, egg, yoghurt, milk, baking powder, lemon juice, and water are kneaded in accordance with the technique, and the dough is shaped into a ring and fried in deep oil (sunflower oil). It is a dessert type which is consumed by dipping it into syrup consisting of sugar, water, and lemon juice (54).

<sup>g</sup> An application has been made to TürkPatent for geographical indication.

<sup>h</sup> Wheat flour, semolina, butter, sugar, egg, and lemon juice are kneaded in accordance with the salt and water mixture technique, and then the dough is shaped and fried in deep oil (sunflower oil). It is a dessert type which is consumed by dipping it into syrup consisting of sugar, water, and lemon juice (55).

<sup>i</sup> Wheat flour is kneaded in accordance with the dry yeast, sugar, and water mixture technique and then shaped and fried in deep oil (sunflower oil). It is a dessert that is consumed by dipping the fried dough in a syrup consisting of sugar and water (56).

carded. Afterwards, 1.5 mL of filtered extract was added to the cartridge. Then 0.5 mL water was used to wash the cartridge. The column eluent was discarded. Then, 1.5 mL of water was loaded onto the cartridge, and the eluant was collected for the second cleanup. Bond Elut Accucat SPE cartridges (Agilent Technologies; Inc. Folsom, CA, USA) were preconditioned first with 2.5 mL MeOH and then with 2.5 mL water. The solvents used for column conditioning were discarded. All of the eluants were loaded with the obtained extract. The first 0.5 mL of the eluate was discarded in this step, and the remaining portion was collected (57). The analysis was performed in triplicates at each level.

#### 2.4. Analysis Conditions

Liquid chromatography was carried out using a UPLC system (Agilent Technologies, model LC-1200 Infinity Series, Englewood, CO, USA) equipped with an autosampler. The used analytical column was a Zorbax Eclipse XDB-C18 (4.6 mm, 150 mm, 5-Micron) (Agilent Technologies, Loveland, CO, USA). The method was operated for 10 min using gradient elution with 0.1% formic acid in water (mobile phase A) and 0.1% formic acid in acetonitril (mobile phase B). Multiple reaction monitoring modes of fragmen-

tation patterns  $m/z$  72 to 55 (acrylamide) and  $m/z$  75 to 58 (acrylamide- $d_3$ ) were employed for quantitation. The flow rate was 0.3 mL/min. LC-MS/MS was operated in the positive ion electrospray at the following conditions: (1) source gas flow: 10 L/min; (2) sheath gas flow: 10 L/min; (3) capillary voltage: 4.0 kV; (4) gas temperature: 350°C; (5) sheath gas temperature: 325°C; (6) nebulizing pressure: 40 psi; and (7) the column temperature: 30°C. The sample injection volume was 10  $\mu\text{L}$ .

#### 2.5. Calibration Curve and Analysis Validation

The calibration solutions were prepared using acrylamide stock solution and acrylamide- $d_3$  stock solution with final concentrations (10, 25, 50, 100, 250, 500, 1000 ng/mL for acrylamide and 100 ng/mL for acrylamide- $d_3$ ). The calibration curve was created by adding 100 ng/mL acrylamide- $d_3$  into seven different concentrations (10 - 1000 ng/mL) using acrylamide stock solution. The prepared solutions were transferred to vials and read in LC-MS/MS device. The calibration graph was plotted based on linear regression analysis of peak area of the acrylamide (Y) versus concentration of acrylamide (X). Acrylamide concentrations in the sample extracts were calculated from

the calibration curve by plotting the area ratio of acrylamide  $m/z$  72 to of acrylamide- $d_3$   $m/z$  75 ions found in the sample. Linearity, LOD, and LOQ were assessed to understand whether the LC-MS/MS method was suitable for analyzing acrylamide in samples. Method performance was evaluated through recovery (R) experiments at different spiking levels (100 and 250 ng/mL) for simit.

### 2.6. Dietary Acrylamide Exposure

In this study, acrylamide exposure from traditional foods was calculated according to the formula stated below.

$$Y = \frac{F \times C_v}{bw}$$

Y is daily exposure ( $\mu\text{g}/\text{kg}$  bw per day), F is the amount of traditional food consumed (g-mL/day),  $C_v$  is acrylamide concentration in traditional foods ( $\mu\text{g}/\text{kg}$ ), bw is body weight (taken as 70 kg).

### 2.7. Risk Assessment

In order to carry out risk characterization, the margin of exposure (MOE) approach was applied. MOE neurotoxic (MOEn) was calculated as the ratio between no observed adverse effect level (NOAEL) (0.2 mg/kg bw per day) and the estimated dietary exposure. MOE carcinogenic (MOEc) was calculated as the ratio between BMDL10 0.31 mg/kg bw per day (0.18 mg/kg bw per day) and the estimated dietary exposure (10).

## 3. Results and Discussion

The  $R^2$  value was found to be higher than 0.995, which means that it is an ideal value. LOD and LOQ values were computed as 3.0 and 10.0 ng/mL, respectively. Acrylamide recovery was 91.6 - 97.2%. RSDs was < 10% for all matrices evaluated. Acrylamide- $d_3$  and acrylamide showed a peak at 5.54 and 5.44 min, respectively. Retention time changes were detected. It was also seen that substances such as lipids in traditional foods could not be completely removed in spite of the clean-up step. Retention time may change slightly for samples which contain a complex matrix (58, 59).

### 3.1. Acrylamide Level of Traditional Foods

It is possible to see traditional meat products, grilled or cooked in a traditional stone oven, in many restaurants' menus in Turkey. As traditional meat products, Adana kebab and lahmacun that were focused in this study are among the world's most well-known flavors (60). Although

the presence of Akçaabat, Akhisar, and İnegöl meat patties on the menu cards is relatively limited compared to Adana kebab and lahmacun, it is possible to find these products in both national and local markets. In addition, franchises of all these products are also very common. Considering these, acrylamide levels of traditional meat products, which are the focus of our research, are shown in Table 1.

The first three products with the highest acrylamide level are stuffed meatball, Adana kebab (grilled), and lahmacun (traditionally baked). The mean acrylamide levels of meat patties (Akçaabat, Akhisar, and İnegöl meat patties) were determined as 43.8  $\mu\text{g}/\text{kg}$ . The lowest acrylamide level product is Rize kavurma (traditionally baked). According to the acrylamide level corresponding to the recommended consumption amount, the order is Adana kebab, stuffed meatballs, and lahmacun. The literature has stated that acrylamide may occur in meat products exposed to high temperatures, especially in breaded fried meat products (2, 61, 62). Few studies have examined the level of traditional meat products in Turkey. In these studies, the acrylamide level was determined as 127 (49 - 250)  $\mu\text{g}/\text{kg}$  in Adana kebab (63), and 68 (< 10 - 203)  $\mu\text{g}/\text{kg}$  (64), 35.8 - 152.8  $\mu\text{g}/\text{kg}$  (65), and 72 - 82  $\mu\text{g}/\text{kg}$  (41) in some meatballs with the unknown geographical indication, respectively. Some researchers found acrylamide levels in meat products in the range of 14 - 222  $\mu\text{g}/\text{kg}$  (66, 67). It is known that the cooking technique in meat products also affects the acrylamide level (2, 67). The highest acrylamide concentration in meat products depending on the cooking technique was determined as 41  $\mu\text{g}/\text{kg}$  for baking, 14  $\mu\text{g}/\text{kg}$  for grilling, 420  $\mu\text{g}/\text{kg}$  for microwaving, 360  $\mu\text{g}/\text{kg}$  for roasting, 298  $\mu\text{g}/\text{kg}$  for deep-frying, and 285  $\mu\text{g}/\text{kg}$  for pan-frying (68, 69).

The inner part of the stuffed meatballs and the outer part of the minced meat have rich starch content, and it is the only product that is deep-fried, analyzed, and included in Table 1. Therefore, formulation and cooking techniques are the main determinants of the highest acrylamide level detected in stuffed meatballs. It is thought that Adana kebab, which contains more ingredients than other products, has a higher level of acrylamide formation because each material contributes to the formation of acrylamide at a different rate. In addition, the combination of Antep lahmacun with thin dough and the presence of bread in the formulation of Akçaabat and İnegöl meat patty may have affected the acrylamide level. The lowest acrylamide level in Rize roast and Akhisar meat patty, which do not contain any ingredients in terms of starch, also indicates the effect of the formulation. Regarding the formulation



contents and literature, it is possible to mention the effect of cooking techniques (deep-frying, barbecue, and traditional baked) on the acrylamide level detected in traditional meat products.

Bread is one of the most indispensable staple food products (70). As it is a cheap and filling food, reached easily, and consumed with many foods, it has a significant place in society's nutrition. About 100 million loaves of bread are produced a day in Turkey. According to the research (Turkey Nutritional Health Survey) conducted by the Republic of Turkey Ministry of Health in 2019, bread consumption was reported to be approximately 83.2, 49.3, and 65.7 kg/year (180 g/day) in men and women aged 15 - 64 and, in all individuals, respectively (44). This ratio is quite high compared to other countries [Belgium: 55 kg/year, Germany: 56 kg/year, France: 57 kg/year, Netherlands: 62 kg/year, Russia: 55 kg/year (71)]. As a kind of bakery products, simit is one of Turkey's most well-known flavors in the world. It is consumed alone or with other foods at any time of the day, especially for breakfast, and is loved by all society. There are many different kinds of Simit depending on the formulation and production techniques in different regions of Turkey (72). Acrylamide levels of bakery products included in this study and protected for geographical indication are shown in Table 2.

The highest acrylamide level and daily acrylamide intake among bread types, depending on bread consumption, belong to Rize baston bread, Vakfıkebir bread, and Gümüşhane bread, respectively. Ankara simit has the highest acrylamide level, and Rize simit has the lowest acrylamide level. The acrylamide level of Samsun simit is very close to that of traditional bread types. In the literature, bread is considered to be one of the important foods in terms of contributing to acrylamide formation and exposure (5, 73, 74). Although bread and simit are very important in the nutrition of Turkish society, it is surprising to see that there are a few studies in the literature. Acrylamide levels of different types of bread in Turkey whose geographical indication is unknown have been detected as 108  $\mu\text{g}/\text{kg}$  (75), 38  $\mu\text{g}/\text{kg}$  (64), 225  $\mu\text{g}/\text{kg}$  (76), 29  $\mu\text{g}/\text{kg}$  (77), and 2.36 mg/kg for all types of simit (78). According to the regulation published by the European Commission in 2017, the acrylamide level in wheat-based soft bread and crispbread should not exceed 50 and 350  $\mu\text{g}/\text{kg}$ , respectively (22). The acrylamide level of bread is detected in the range of < 30 - 160  $\mu\text{g}/\text{kg}$  in Sweden (79), < 20 - 71  $\mu\text{g}/\text{kg}$  in Brazil (14), 56.9  $\mu\text{g}/\text{kg}$  in Romania (80), 42 - 49  $\mu\text{g}/\text{kg}$  in Iran (81), < LOQ-237  $\mu\text{g}/\text{kg}$  in Croatia (82), 31 - 454  $\mu\text{g}/\text{kg}$  in Italy (83).

While the acrylamide level in the literature has a very

wide range in terms of bread, this range is relatively limited, and the acrylamide level is lower in our study. The average acrylamide levels of bread were found to be very close to each other. It is estimated that this situation is particularly related to the sourdough used in the production of Vakfıkebir and Gümüşhane bread and the fermentation process because acrylamide level decreases due to the decrease in the sugar ratio in the environment with fermentation. It is estimated that the difference in acrylamide level detected in simit is due to the formulation. Despite using similar ingredients in the production of each simit, sesame, and molasses are the distinguishing features. The fact that Rize simit is sesame-free is the reason why the acrylamide level was measured at a very low level compared to others. Therefore, it can be accepted as an example of this situation.

Pastries have a special place in Turkish cuisine culture. The love of pastry foods in society has led to forming a very rich dessert culture. Among these desserts, baklava is one of the most well-known flavors in the world (84, 85). Halka, lokma, and tulumba desserts are inexpensive, and takeaway, which are quite common as street food. There are three steps in dessert production in general; first, the specific formulation of the dessert is prepared, and its traditional shape is given, second, the dough is shaped and fried in oil or baked in the oven; third, it is served with or without syrup depending on the type of dessert. Desserts are mostly consumed with nuts (peanuts, hazelnuts, walnuts). Acrylamide levels of traditional desserts are shown in Table 3.

The first three desserts with the highest acrylamide level are halka, tulumba, and lokma, and the one with the lowest acrylamide level is Laz böreği. The average acrylamide levels of künefe and baklava, which are the most common and consumed, ranged from 31.3 to 40.6  $\mu\text{g}/\text{kg}$ . Although the acrylamide level of kaymaklı ekmek kadayıfı (crumpets in thick syrup) has an average value, it stands out in acrylamide intake considering the portion amount. It is known that high levels of acrylamide are formed by heat treatment of carbohydrate-rich foods (5). In traditional Turkish desserts (geographical indication unknown), acrylamide level was detected in the range of < 10 - 220  $\mu\text{g}/\text{kg}$  in künefe (64, 78), and 25 - 1,580  $\mu\text{g}/\text{kg}$  in baklava (78, 86), < 10 - 2,470  $\mu\text{g}/\text{kg}$  in tulumba dessert (64, 78, 86), and 75 - 115 and 111  $\mu\text{g}/\text{kg}$  in halka and lokma dessert (86), respectively.

Traditional desserts can be divided into 2 groups according to their cooking techniques. Künefe, baklava, Laz böreği and kaymaklı ekmek kadayıfı are cooked in the oven, while halka, tulumba, and lokma desserts are fried

in deep oil. According to our research findings, acrylamide levels of desserts deep-fried (approximately 3 - 5 min at 230 - 250°C) were found to be significantly higher than those baked in the oven (20 - 25 min. at approximately 220 - 230°C). In addition, the ingredients put into the dough in künefe, baklava, and laz böreği constitute a significant part of the final product, while the halka, tulumba, and lokma desserts do not contain any internal material, and the final product has a more starch content. From this point of view, it can be mentioned that both the cooking technique and the formulation have an important effect on the acrylamide level. This information is also compatible with the literature.

### 3.2. Acrylamide Exposure Risk Assessment

Considering the data in Tables 1 - 3 and consumption trends of these foods, good, average, and bad scenarios were developed, and acrylamide exposures of individuals were estimated (Table 4). Foods in Table 1 are accepted as the main course. For good and bad scenarios, the foods with the lowest and highest acrylamide levels were selected in each product group. Acrylamide exposure was calculated by taking the average acrylamide level of each product group in the average scenario. Accordingly, daily acrylamide exposure was calculated as 0.20, 0.53, and 0.97  $\mu\text{g}/\text{kg}$  bw per day for good, average and bad scenarios, respectively. JECFA estimated acrylamide exposure to be 1 and 4  $\mu\text{g}/\text{kg}$  bw per day, respectively, for both the general population and high-exposure consumers (31). JECFA and FAO/WHO stated in 2011 that the acrylamide neurotoxic NOAEL value in mice was 0.2 mg/kg bw per day (10). The acrylamide exposure value calculated for the average-case scenario in the study is very close to similar studies (5, 87, 88) in Colombia (0.52  $\mu\text{g}/\text{kg}$  bw per day), the European Union (0.50  $\mu\text{g}/\text{kg}$  bw per day), Germany (0.50  $\mu\text{g}/\text{kg}$  bw per day), and the Netherlands (0.48  $\mu\text{g}/\text{kg}$  bw per day). The calculated acrylamide exposure value for the good-case scenario is higher than the mean value in similar studies in Croatia (0.16  $\mu\text{g}/\text{kg}$  bw per day), Iran (0.15  $\mu\text{g}/\text{kg}$  bw per day), and Japan (0.17  $\mu\text{g}/\text{kg}$  bw per day) (82, 89). The acrylamide exposure value calculated for the bad-case scenario is relatively high compared to other studies and is close to the mean value determined by JECFA (31).

According to good, average and bad scenarios, the MOEn value was calculated as 1,000, 377, and 206, and the MOEc value as 900, 339, and 185, respectively. Joint FAO/WHO Expert Committee on Food Additives calculated that the minimum margin of exposure (MOE) for acrylamide was 200 for mean dietary exposure, and 50 for high dietary exposure. BMDL10 value under 310 (180) or 78 (45)

for acrylamide may be of concern in carcinogenic or neurotoxic terms (10).

## 4. Conclusion

In this study, the acrylamide level of 20 different traditional foods of different geographies in Turkey was identified, and a risk assessment was carried out. According to acrylamide levels, traditional foods are listed as desserts > meat products > bakery products. This situation is thought to be related to the type of raw material and different process conditions. Acrylamide exposure calculated according to different scenarios is generally higher than other studies. However, the average MOE values are unlikely to have a negative effect in terms of both neurotoxic and carcinogenic. Our research is the most comprehensive study to determine acrylamide levels in traditional foods in Turkey. Carrying out more studies on this issue in Turkey, which is rich in terms of traditional foods, is significant for reducing the number of acrylamide levels and preparing strategies to estimate exposures. Based on this, it is believed that the results will be a good source for similar studies. Additionally, this research undoubtedly has some limitations. First, it is very difficult to obtain a standard quality product depending on the different formulation, and cooking methods used in the production of traditional foods. Therefore, traditional foods in our research may differ in appearance, textural, nutritional, and sensory properties. In addition, the portion amounts of the products may differ significantly according to the restaurant. It should be noted that both of these conditions can directly affect acrylamide levels and exposure.

## Footnotes

**Authors' Contribution:** Author contributions are equal. All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version.

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**Table 4.** Estimating the Acrylamide Exposure<sup>a</sup>

Scenarios	Breakfast (Simit); $\mu\text{g}$	Lunch (Main Course); $\mu\text{g}$	Dinner (Main Course + Dessert); $\mu\text{g}$	All Day (Bread); $\mu\text{g}$
<b>Good</b>	0.75 (Rize simit)	3.1 (Kavurma)	3.1 + 1.17 (Akhisar meat patty + Laz böreği)	6.15 (Gümüşhane bread)
<b>Average<sup>b</sup></b>	3.20	7.52	7.52 + 12.15	6.65
<b>Bad</b>	6.35 $\mu\text{g}/60\text{ g}$ (Ankara simit)	12.86 (Stuffed meatball)	17.70 + 37.0 (Adana kebab + Halka dessert)	7.30 $\mu\text{g}/60\text{ g}$ (Baston bread)

<sup>a</sup> Body weight (bw) is accepted as 70 kg.<sup>b</sup> Each product group is averaged.

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