Determination of Acrylamide Levels in Infant Formulas and Baby Biscuits Sold in Turkey

Burhan Başaran [1,*] , **Ferid Aydın** [2]

1   Department of Travel, Tourism and Recreation Services, Ardeşen Vocational School, Recep Tayyip Erdogan University, Rize 53100, Turkey; burhan.basaran@erdogan.edu.tr (B.B.);
2   Department of Food Engineering, Faculty of Agriculture, Ataturk University, Erzurum 25100, Turkey; feray@atauni.edu.tr (F.A.);
*   Correspondence: burhan.basaran@erdogan.edu.tr (B.B.);

Abstract: Babies are more vulnerable to both environmental and dietary contaminants compared to adults. Hence, the safety of baby foods deserves to be given top priority. In this study, the acrylamide level of 56 different baby foods (6 infant formula and 10 baby biscuit brands) which are widely consumed and have a high level of awareness in Turkey, has been determined and evaluated with LC-MS/MS. Acrylamide level was measured in the range of <LOQ–578 µg kg^{-1} in infant formulas and 12.3–1,270 µg kg^{-1} in baby biscuits. The average acrylamide level in baby foods consumed by 0-5, 6-12, and >12 months children were determined to be 45.1, 62.5, and 88.6 µg kg^{-1}, respectively. The average acrylamide level of baby biscuits is 233 µg kg^{-1}. The average acrylamide level corresponding to 1 portion/scale in infant formulas and biscuits was calculated as 0.30 µg/30 mL and 4.72 µg/20 g, respectively. Research results can be used in acrylamide reduction and acrylamide exposure risk assessment studies.

Keywords: infant formula; baby biscuit; acrylamide; dietary acrylamide exposure; LC-MS/MS.

1. Introduction

World Health Organization advises that infants need to be exclusively breastfed for the first 6 months and receive complementary foods in addition to breast milk until 24 months. In contrast, the rate of breastfeeding after childbirth is dropping dramatically worldwide [1]. Undoubtedly, many internal and external factors affect the mother's breastfeeding behavior [2]. In addition to the behavioral change caused by all these factors, mothers also provide other foods (especially packaged baby foods) for their children with or without breast milk, depending on their development [3].

Today, many companies are operating globally in the baby food industry [4]. It is possible to find many baby foods formulated to develop 0–6, 6–12, and >12 months children and enriched with various vitamins and minerals [5]. Baby foods are often preferred because of their easy accessibility and the practical preparation process [3]. However, it is also known that parents are seriously concerned about the composition, production, and safety of these foods [6]. Acrylamide is one of the compounds that cause this concern.

Acrylamide (2-propenamide, CAS No. 79-06-1) is a colorless, odorless, crystalline powder compound with low molecular weight (71.08 kDa), easily soluble in water, ethanol,

and acetone [7]. Acrylamide, which is highly used in areas such as dams, tunnels, water treatment, paper, and textiles [8], was first revealed in 2002 by Tareke *et al.* (2002) [9].

Acrylamide has a genotoxic and neurotoxic effect [10], and it was identified as a probable carcinogen for humans (Group 2A) by the International Agency for Research on Cancer (IARC) in 1994 [11]. Although there are findings in the literature that there is a positive relationship between acrylamide intake based on nutrition and cancer [12,13], studies indicating no relationship are also available [14,15]. Uncertainties regarding the formation process in acrylamide foods persist [16]. It has been stated that acrylamide is formed at high levels with heat treatment, especially in foods rich in asparagine amino acids and reducing sugar [9,17]. Although Maillard Reaction is accepted as the main mechanism in acrylamide formation, studies prove that acrolein, carnosine, B-alanine, aspartic acid, and pyruvic acid are transformed into acrylamide by various reactions [18,19].

The European Commission and the Joint FAO/WHO Expert Committee on Food Additives stated that there is very little information on the level of acrylamide in foods and its formation, and advised to monitor and report systematically the exposure to foods containing high acrylamide and consumption of these foods [20–22]. The European Food Safety Authority (EFSA) conducted a study in the European Union in 2015 and reported that French fries, potato chips, bread, biscuits, breakfast cereals, coffee (dry), infant formula, and baby foods (processed cereal-based) contain 308 (303-313) μg kg^{-1}, 389 (388-389) μg kg^{-1}, 42 (36-49) μg kg^{-1}, 265 (261-269) μg kg^{-1}, 161 (157-164) μg kg^{-1}, 522 (521-523) μg kg^{-1}, 14 (3-26) μg kg^{-1}, and 73 (70-76) μg kg^{-1} acrylamide, respectively. In the same study, it was stated that acrylamide exposure (P95) based on nutrition for infants, toddlers, other children, adolescents, adults, the elderly, and the very elderly changed in the range of 1.4-2.5, 1.4-3.4, 1.4-3.2, 0.9-2.0, 0.8-1.3, 0.7-1.0 $\mu g/kg$ bw per day, respectively [23]. Acrylamide levels in infant formula and baby foods are lower than those in other foods. However, the exposure to consumers (infants, toddlers) who target these foods is surprisingly higher than other consumer groups. Many researchers have also stated that children are exposed to acrylamide more because their body weight is lower than others and the variety of nutrition is limited [24–26].

In Turkey, approximately 1.2 million babies are born annually, and about 6.5 million children at the age ranging between 0 and 4 live [27]. It is stated that baby food consumption is at a low level in Turkey compared to other countries. However, the baby food market has almost always shown double-digit growth in the last 10 years, and it is expected to further grow in the future [28]. Studies have been conducted to determine acrylamide levels in baby food in Turkey [29–33]. However, the infant foods examined in the studies do not provide sufficient information in terms of both the number of samples and product details. This situation is mostly observed in other studies. For instance, the studies do not explain adequately which age group corresponds to infant formulas or acrylamide level of one portion for infant formula, and biscuits are not calculated according to the information given by the manufacturer on the packaging. It should be remembered that these data losses might adversely affect studies of dietary acrylamide exposure. Besides, it seems that recent studies were conducted in Turkey in 2013 and 2014. As it is known, the food sector is a very dynamic sector that constantly renews itself in the light of new information. Therefore, the period covering the last 7 years is sufficient for the occurrence of important changes in areas such as formulation and production technique of products.

In light of the current data in the literature, the exposure that starts with breast milk and infant formulas in the first months of the baby continues throughout life with changing

nutritional habits according to the development of the individual. Therefore, knowing how and in what quantity and frequency we consume are closely related to public health. The aim of this study is to determine acrylamide levels in the consumption of different brands of baby food in Turkey that has a high level of awareness and consumption.

2. Materials and Methods

2.1. Samples.

In this study, infant formulas (6 popular brands) and baby biscuits (10 different popular brands) which are sold and consumed in Turkey, are examined. Infant formulas in Turkey are numbered as 1, 2, and 3 according to different age groups (0–6, 7–12, and >12 months). Total 56 baby foods (36 infant formulas and 20 baby biscuits) in their original packaging, including 2 products with different production dates representing each brand and number, were purchased from markets.

2.2. Reagents and apparatuses.

Acrylamide (99%) and acrylamide-d_3 were provided by Sigma-Aldrich (St. Louis, MO). Maxi-Spin filter tubes (0.45 μm PVDF) and HPLC grade acetonitrile, methanol, formic acid, n-hexane were obtained from ISOLAB (Wertheim, Germany). Oasis HLB (6 mL, 200 mg) and Bond Elut-Accucat (200 mg, 3 mL) SPE cartridges were supplied by Waters (Milford, MA) and Agilent Technologies (Inc. Folsom, CA, USA), respectively.

Liquid chromatography was carried out using a UPLC system (Agilent Technologies, model LC-1200 Infinity Series, Englewood, CO, USA) equipped with an autosampler. The Allegra X-30R centrifuge (Beckman Coulter, Palo Alto, CA) was equipped with a C0650 head. The analytical column used was a Zorbax Eclipse XDB-C18 (4.6 mm, 150 mm, 5-Micron) (Agilent Technologies, Loveland, CO, USA).

2.3. Stock and working solution.

Stock and working standards of acrylamide (99%) and acrylamide-d_3 were prepared in HPLC-grade water with 0.1% formic acid. Working standard solutions were prepared by diluting the stock solution of acrylamide. Stock and working solutions are stored at 4°C until use.

2.4. Sample extraction.

Samples of 1 g were weighed into a 50 mL centrifuge tube, and 9 mL water, 1 mL (100 ng mL^{-1}) of acrylamide-d_3, and 5 mL hexane was added. The centrifuge tubes were shaken or vortexed for 3 min to mix contents. The tubes were centrifuged at 9000 rpm for 15 min. A pipet was used to transfer a 5 mL aliquot of the clarified aqueous layer to a filtration tube through a Maxi-Spin 0.45 μm PVDF. The tubes were centrifuged at 6000 rpm for 5 min. Oasis HLB cartridges and A Bond Elut Accucat SPE cartridges were preconditioned with first 3.5 and 2.5 mL MeOH and then 3.5 and 2.5 mL water, respectively. The solvents used for column conditioning were discarded. 1.5 mL of filtered extract was added to the Oasis HLB cartridge, then 0.5 mL water was used to wash the cartridge, and column eluent was discarded. Then 1.5 mL of water was loaded into the Oasis HLB cartridges, and the eluant was collected from the

SPE cartridge. The first 0.5 mL of the eluent was discarded, and the remaining 1 mL of the extract was transferred to a vial [34].

2.5. Analysis conditions.

LC-MS/MS analysis conditions are shown in Table 1.

Table 1. LC-MS/MS analysis conditions

Mobile phase A	0.1% formic acid in water
Mobile phase B	0.1% formic acid in acetonitrile
Mobile phase flow rate	0.3 mL min^{-1}
Source gas flow	10 L min^{-1}
Sheath gas flow	10 L min^{-1}
Nebulizing pressure	40 psi
Capillary voltage	4000 V
Gas temperature	350°C
Sheath gas temperature	325°C
Column temperature	30°C
Injection volume	10 μL

2.6. Quality control.

The linearity parameters, the limit of detection (LOD) and the limit of quantitation (LOQ), repeatability, reproducibility, and average recovery parameters were calculated to ensure the LC-MS's appropriateness/MS method for the analysis of acrylamide in baby foods. Linearity of the method was evaluated by building at seven standard solutions (10, 25, 50, 100, 250, 500, 1000 ng mL^{-1}) with 100 ng mL^{-1} of acrylamide-d$_3$. In the calculation of the coefficient of determination (R^2), linear regression analysis was utilized. The limit of detection (LOD) and the limit of quantitation (LOQ) values were calculated. Method performance was evaluated by means of recovery experiments at different spiking levels (30 and 100 ng mL^{-1}).

3. Results and Discussion

3.1. Method verification.

The calibration data fitted a linear regression model with a good value (R^2: 0.9997209) of coefficient of the range of 10–1000 ng mL^{-1} (Figure 1). The LOD and the LOQ values were calculated as 3.0 and 10.0 ng mL^{-1}, respectively.
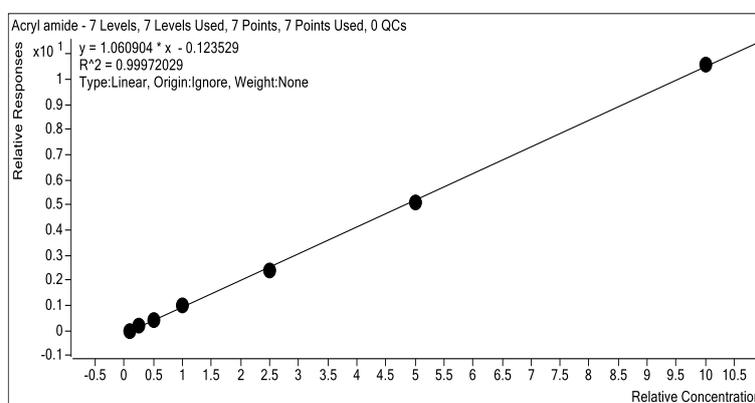

Figure 1. Calibration graph for baby foods spiked with acrylamide and acrylamide-d$_3$.

The method was verified for two food matrices. Acrylamide recovery was 93.1–97.4% and 95.3–98.2% for baby food and baby biscuits, respectively. RSD was <7% for all matrices evaluated (Table 2). The results were similar to other studies in the literature [35,36]. Thus, this method can be applied to the analysis.

Table 2. Average recovery values (R%) and RSD (%) obtained for acrylamide.

Food	Spike level (ng g⁻¹)	Repeatability (R±RSD %)	Reproducibility (R±RSD %)
Infant formula	30	93.1 ± 4.3	97.4 ± 4.7
	100	90.5 ± 2,8	95.2 ± 4.3
Baby biscuit	30	95.3 ± 3.5	98.2 ± 5.3
	100	94.6 ± 4.1	99.8 ± 6.2

The chromatograms of the fragment ions m/z 58.20 and 44.10 of the internal standard and m/z 55.10 and 44.10 for acrylamide in baby foods were used for quantification (Figure 2). Figure 3 illustrated the acrylamide chromatography of selected baby food. The retention time was determined to be 5.45 min.
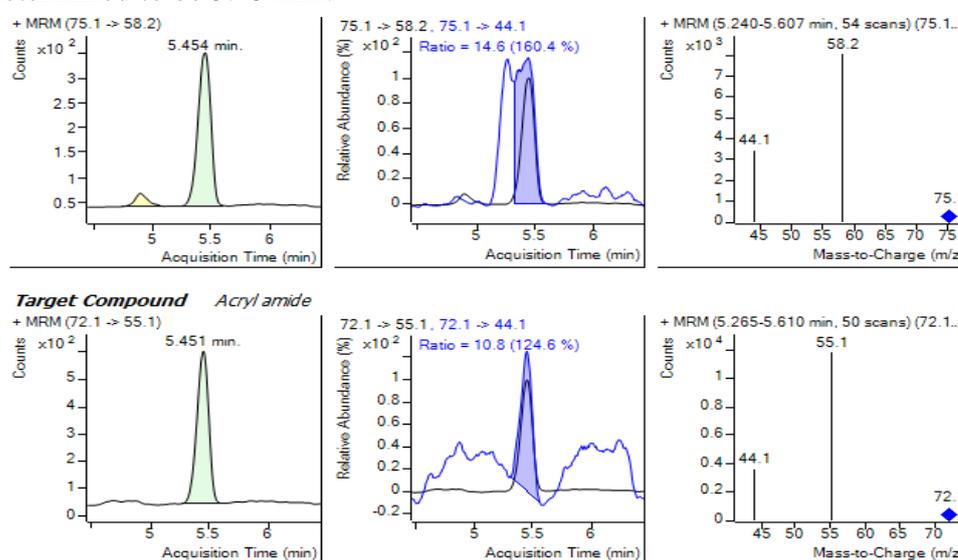

Figure 2. LC-MS/MS chromatogram of (**a**) acrylamide-d₃ fragment ions m/z 58.20 and 44.10; (**b**) acrylamide fragment ions m/z 55.10 and 44.10 in infant formulas.
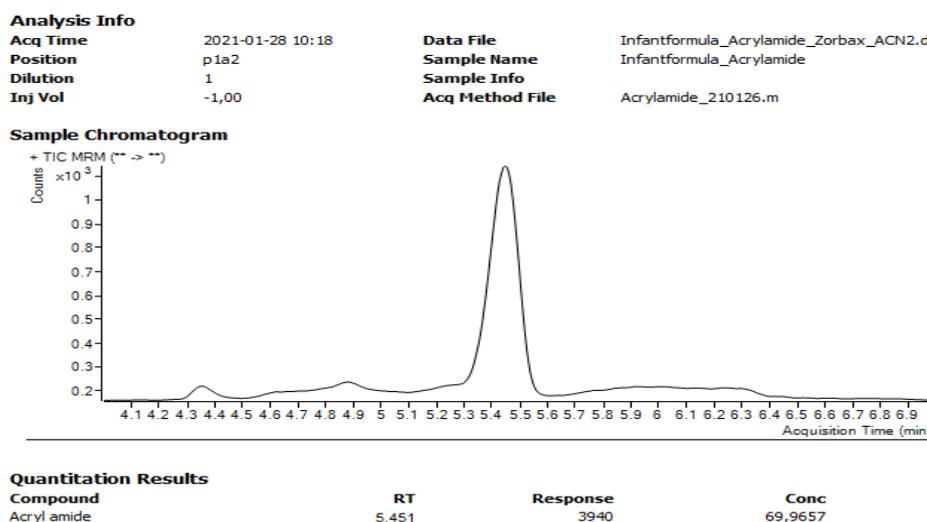

Figure 3. The acrylamide chromatography of selected infant formula.

3.2. Acrylamide levels of baby foods.

Acrylamide levels and recommended 1 scale for consumption to the corresponding estimates in 6 brands and 18 different content in infant formula in Turkey are shown in Table 3.

Table 3. Acrylamide levels of infant formulas.

Age groups/Brands	N	Range (µg kg⁻¹)	Mean µg kg⁻¹	µg 30 mL⁻¹ᵃ	Noteᵇ
0-6 months (Number 1)					
Brand 1	2	14.7-127	48.0	0.21	4.30 g
Brand 2	2	11.8-61.0	31.2	0.14	4.50 g
Brand 3	2	<LOQ-23.8	13.2	0.06	4.50 g
Brand 4	2	15.4-135	68.8	0.29	4.30 g
Brand 5	2	<LOQ-162	54.2	0.25	4.60 g
Brand 6	2	<LOQ-143	55.1	0.25	4.60 g
		Mean	*45.1*	*0.20*	
7-12 months (Number 2)					
Brand 1	2	<LOQ -140	65.3	0.29	4.50 g
Brand 2	2	<LOQ -139	75.8	0.36	4.80 g
Brand 3	2	13.6-55.8	25.6	0.12	4.60 g
Brand 4	2	18.2-228	89.7	0.40	4.50 g
Brand 5	2	25.6-188	69.5	0.34	4.85 g
Brand 6	2	23.8-142	49.3	0.23	4.60 g
		Mean	*62.5*	*0.29*	
>12 months (Number 3)					
Brand 1	2	13.4-98.0	52.7	0.21	4.00 g
Brand 2	2	44.6-207	103	0.49	4.80 g
Brand 3	2	<LOQ-91.3	31.8	0.15	4.90 g
Brand 4	2	21.9-578	251	1.15	4.60 g
Brand 5	2	14.6-102	54.3	0.27	4.95 g
Brand 6	2	19.2-70.4	40.4	0.18	4.60 g
		Mean	*88.9*	*0.41*	

N: Number of samples, ᵃ One scale is 30 mL – the information given by the producer on package,
ᵇ It is the amount that should be put on 1 scale according to the information given on the package by the manufacturer.

The acrylamide level in infant formula number 1 (0–6 months) is in the range of <LOQ–162 µg kg⁻¹, and the average acrylamide level of all brands was found to be 45.1 µg kg⁻¹. The average highest acrylamide level belongs to Brand 4 (68.8 µg kg⁻¹). The average acrylamide level corresponding to 1 scale is 0.20 (0.06–0.29) µg kg⁻¹ (Table 3).

Brands 1 and 2 have the highest average acrylamide level in number 2 infant formula (7–12 months). The average acrylamide level of all products is 62.5 µg kg⁻¹. The average acrylamide level corresponding to 1 scale is 0.29 (0.12–0.40) µg kg⁻¹and the lowest acrylamide level was determined in Brand 3 (Table 3).

In infant formula, number 3 (>12 months), acrylamide level was in the range of <LOQ–578 µg kg⁻¹, and mean acrylamide level was determined as 88.9 µg k kg⁻¹. Brands with the highest and the lowest average acrylamide levels are Brand 4 (251 µg kg⁻¹) and Brand 3 (31.8 µg kg⁻¹), respectively. The average acrylamide level corresponding to 1 scale is 0.41 (0-15–1.20) µg kg⁻¹ (Table 3).

When all the data in Table 3 were evaluated together, acrylamide level in infant formula was measured in the range of <LOQ–578 µg kg⁻¹. The average acrylamide level of all infant formulas was measured as 69.9 (13.2–251) µg kg⁻¹. 1 scale corresponding to the acrylamide level was calculated as 0.30 (0.06–1.20) µg kg⁻¹. The acrylamide level of 8 (11.1%) of the 72 samples analyzed were found below the LOQ value. As children get older, the acrylamide level

in the infant formula they also consume increases. When acrylamide levels corresponding to 1 scale are compared, similarly, acrylamide level increases again in direct proportion to age. Undoubtedly, one factor contributing to this situation is the increase in the amount of infant formula added to 1 scale depending on age. Infant formulas with the lowest and the highest acrylamide levels are the same for all age groups and are Brand 3 and 4, respectively. It was not possible to reach a study showing the level of acrylamide in infant formulas consumed by children in Turkey. Similarly, infant formulas have been generally studied in other studies. Therefore, the comparison of the results obtained in this study with other studies was made in light of the data obtained for all infant formulas (all age groups) (Figure 5).
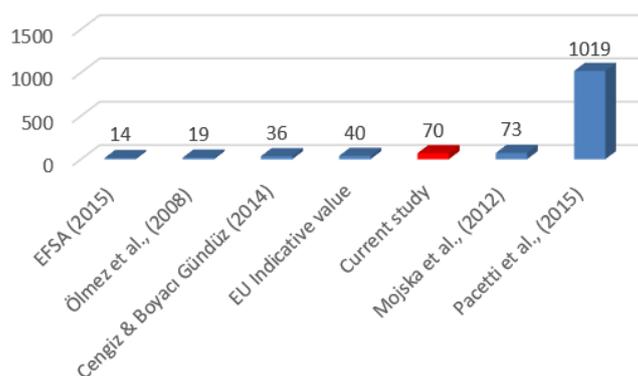

Figure 5. Average acrylamide levels in infant formulas in current research and other studies ($\mu g \ kg^{-1}$).

It has been found that there are only two studies on the levels in infant formulas sold in Turkey, and the number of studies is quite limited. In these studies, acrylamide level was determined to be 19 $\mu g \ kg^{-1}$ (8 samples) and 36 $\mu g \ kg^{-1}$ (7 samples) by Ölmez *et al.* (2008) and Cengiz and Boyacı Gündüz (2014), in infant formulas of unknown age groups [30,32]. The European Commission (EC 2017/2158) determined the indicative acrylamide value in infant formulas to be 40 $\mu g \ kg{-1}$ [20]. In other studies, the acrylamide level was found as $<5 \ \mu g \ kg^{-1}$ by Keramat *et al.* (2011), 73 (32–312) $\mu g \ kg^{-1}$ by Mojska *et al.* (2012), and in one scale it was 0.32 (0.13–1.38) $\mu g \ kg^{-1}$, *Pacetti et al.* (2015) determined as 1.019 (<LOQ–1.821) $\mu g \ kg^{-1}$, EFSA (2015) as 14 (3–26) $\mu g \ kg^{-1}$ and Lambert *et al.* (2018) determined it as 0.60–2.90 $\mu g \ kg^{-1}$ [23,25,36–38].

The acrylamide levels and estimated values corresponding to the recommended consumption in baby biscuits that are commercially available in Turkey with 10 brands and 20 different content are shown in Table 4.

Table 4. Acrylamide levels of baby biscuits.

Brands	N	Range ($\mu g \ kg^{-1}$)	Mean $\mu g \ kg^{-1}$	1 portion[c]	Note
Brand 1	2	32.7-179	96.7	1.93	From 4 months
Brand 2	2	78.2-1,270	608	12.16	From 6 months
Brand 3	2	17.5-176	91.9	1.84	From 6 months
Brand 4	2	26.1-299	185	3.69	From 6 months
Brand 5	2	24.2-121	75.1	1.50	From 6 months
Brand 6	2	12.3-840	250	6.25	From 6 months
Brand 7	2	39.6-510	193	4.83	From 6 months
Brand 8	2	52.0-1,064	431	7.00	From 6 months
Brand 9	2	59.3-131	89.4	1.79	From 6 months
Brand 10	2	63.8-1,020	311	6.21	From 6 months
		Mean	*233*	*4.72*	

N: Number of samples,

[c] One portion includes 4-5 biscuits (20 g) – the information given on the package by the manufacturer.

The acrylamide level in baby biscuits has a very wide range from 12.3 to 1,270 µg kg^{-1}. The average acrylamide level of all baby biscuits was determined to be 233 µg kg^{-1}. The first three baby biscuit brands with the highest average acrylamide level are Brand 2 (608 µg kg^{-1}), Brand 8 (431 µg kg^{-1}), and Brand 10 (311 µg kg^{-1}), respectively. The lowest average acrylamide level is Brand 5 (75.06 µg kg^{-1}). The acrylamide level corresponding to 1 portion is between 1.50–12.2 µg kg^{-1}, and the average acrylamide level is calculated as 4.72 µg kg^{-1}. The comparison of the average acrylamide level detected in baby biscuits in this study with other studies is shown in Figure 6.
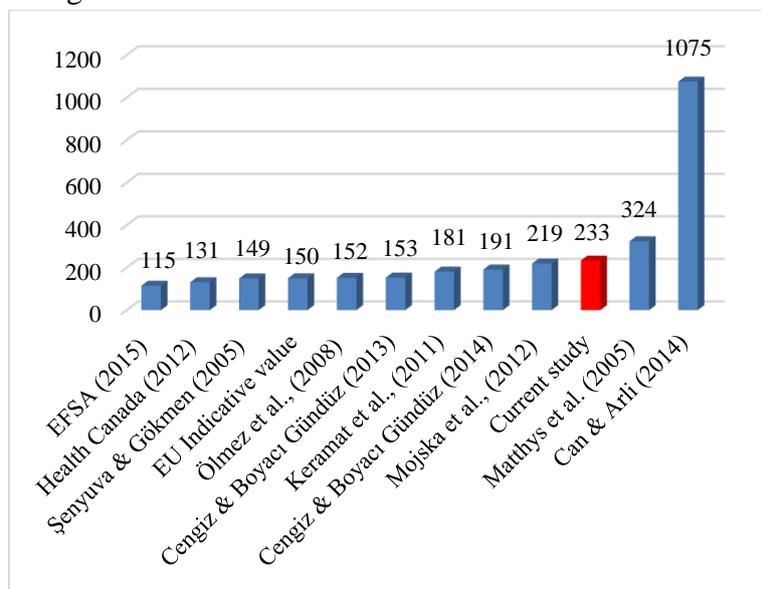

Figure 6. Average acrylamide levels in baby biscuits in current research and other studies (µg kg^{-1}).

The acrylamide level in baby biscuits sold in Turkey was determined to be 149 µg kg^{-1} (3 samples) by Şenyuva and Gökmen (2005), 152 µg kg^{-1} (3 marks–14 samples) by Ölmez *et al.* (2008), 153 µg kg^{-1} (3 brands–33 samples) and 191 µg kg^{-1} (10 samples) by Cengiz and Boyacı Gündüz (2013; 2014), respectively and 1,075 (490–1,660) µg kg^{-1} by Can and Arli (2014) [29–33]. The European Commission (EC 2017/2158) has determined the indicative acrylamide value for baby biscuits consumed by infants and young children to be 150 µg kg-1 [20]. In other studies, Matthys *et al.* (2005) determined it to be 324 (225–1,217) µg kg^{-1}, Keramat *et al.* (2011) 181 µg kg^{-1}, Health Canada (2012) 131 (119–139) µg kg^{-1}, Mojska *et al.* (2012) 219 (37–516) µg kg^{-1}, and in one portion (25 g) it was 5.50 (0.93–12.90) µg kg^{-1}, Michalak *et al.* (2013) 10.8–61.2 µg kg^{-1} (cereal-based baby foods), EFSA (2015) 115 µg kg^{-1} and Lambert *et al.* (2018) 11–17 µg kg^{-1} (cereal-based baby foods) [23,25,37–41].

4. Conclusions

In this research, we focused on infant foods, which have an important place in the nutrition of the most vulnerable individuals, namely children. The acrylamide level has a very wide range of baby food and biscuits. In general, a relatively higher acrylamide level was determined in the study compared to other studies, especially in the indicative values determined by the European Commission in both baby food and baby biscuits. This situation is related to the type, amount, and process conditions of the raw material used in the product formulation. Our research is one of the most comprehensive studies in Turkey that determine and assess the acrylamide level in baby foods and biscuits. The study is considered a good resource for risk assessment studies of acrylamide exposure from infant foods. More studies

need to be conducted, including other types of baby foods in Turkey, where the baby food market constantly grows.

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Conflicts of Interest

The authors declare no conflict of interest.

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