

ASSESSMENT OF THE ACRYLAMIDE LEVEL OF CEREAL-BASED PRODUCTS FROM ROMANIA MARKET IN ACCORDANCE WITH COMMISSION REGULATION (EU) 2017/2158

ADRIANA LAURA MIHAI, MIOARA NEGOIȚĂ*, GABRIELA-ANDREEA HORNET

National Research & Development Institute for Food Bioresources, IBA Bucharest, 6 Dinu Vintilă Street, District 2, 021102, Bucharest, Romania

*corresponding author: mioaranegoita@yahoo.com

Received on 27 March 2020

Revised on 1 May 2020

Abstract

This study reports the acrylamide (AA) content of some cereal-based products purchased from different Romanian suppliers. The AA level was determined by using gas chromatography tandem mass spectrometry (GC-MS/MS) in SRM mode. The analytical method was characterized by a high degree of sensitivity (limit of detection (LOD) = 1.23 µg/kg, and limit of quantification (LOQ) = 3.70 µg/kg).

A total of 46 food samples of bread, biscuits, and similar products (doughnuts, cakes, cookies etc.) were analysed. The results showed that the AA level varied between food samples, for soft bread (5.63 – 85.05 µg/kg), biscuits (8.55 – 548.80 µg/kg), crackers (13.92 – 167.58 µg/kg) and for similar products (12.57 - 99.01 µg/kg) which were lower than the benchmark levels set by the Commission Regulation (EU) 2017/2158. The highest AA content (548.80 µg/kg) was found for biscuits, while the lowest AA content (5.63 µg/kg) was obtained for wheat-based bread. From the analysed products, 4 biscuit samples exceeded the benchmark levels of 350 µg/kg established by European Union.

Keywords: acrylamide, biscuits, bread, cereal-based products, GC-MS/MS

Introduction

Cereal-based products are an important source of fibres in the diet in many countries, contributing to human health. From the cereal products, bakery products are the most important category. During the baking process, the Maillard reaction takes place by interaction between reducing sugars and asparagine, an amino acid found in plant-based products, like this being formed acrylamide (AA). AA is a

chemical compound found when carbohydrates and protein-rich foods are subjected to temperatures higher than 120°C and in low moisture conditions.

Since 2002, when it was discovered in food, many studies have been made to determine the human exposure to AA and its effect on human health. AA was categorized as a member of the group 2A substances by the International Agency for Research on Cancer (IARC, 1994), which means that is probably carcinogenic to humans. AA poses a serious risk to humans having cytotoxic effect producing cellular oxidative stress, apoptotic, having anti-proliferative activity (Kacar *et al.*, 2019), affecting the reproductive system (AL Karim *et al.*, 2015; Matoso *et al.*, 2019). Due to this impact on human health, EFSA experts established a benchmark dose lower confidence limit (BMDL₁₀) of 0.17 mg/kg body weight (b.w.)/day for tumours incidence and 0.43 mg/kg b.w./day, respectively for other potential adverse consequences, as neurological changes and effects on reproductive system. It was also set a margin of exposure (MOE) which indicate the level of health concern, these values varying between 425 for average adult consumer and down to 50 for high consuming toddlers (EFSA, 2015). The exposure of young people is higher than that of adults, in particular, the exposure of toddlers is in the highest range. In addition, The Joint FAO/WHO Expert Committee on Food Additives stated that children dietary exposure to AA were about twice than those of adult consumers, when expressed on a b.w. basis (FAO/WHO, 2011).

It was reported that the mean AA exposure in Europe was estimated to range from 0.4 to 0.6 µg/kg b.w./day for adults, 0.4– 0.9 µg/kg b.w./day for adolescents, 0.5– 1.3 µg/kg b.w./day for infants and 0.9– 1.9 µg/kg b.w. per day for toddlers (EFSA, 2015).

Based on the monitored results, the European Commission has implemented the Regulation 2017/2158 to establish mitigation measures and benchmark levels for the reduction of the presence of acrylamide in food. These benchmark levels are not safety values but performance indicators used to verify the effectiveness of the mitigation measures applied for AA reduction in different food. It should be established as low as reasonably achievable with the application of all relevant mitigation measures.

Also, the European food and drink industries together with national authorities of the European Union proposed a toolbox, in which was presented the pathways of acrylamide formation and recommended some mitigation strategies (Food Drink Europe, 2019).

The AA level of cereal-based products is influenced by the type of cereal flour used (refined/wholegrain), the ingredients, the formulation recipe, the dough final moisture content, and also thermal processing (baking temperature and time) play an important role (Claus *et al.*, 2008, Capuano *et al.*, 2009, Przygodzka *et al.*, 2015, Negoită *et al.*, 2017a). In the last years, consumers demand for healthier products lead to the use of pseudo-cereals in the recipe, which are either mixed with traditional cereals or are used to replace them totally. From the nutritional point of view, the final product is better than the traditional one but concerns are raised regarding the AA content. In case of cereal-based products, free asparagine

is the determinant factor of AA formation. Žilić *et al.* (2020) studied the effect of various wholegrain flours with different content of free asparagine on the AA content of biscuits baked at different condition and showed that the AA level of the final products is correlated with the initial asparagine content of the cereal flours used and the baking time. Compared with refined wheat flour, when the wholegrain bread wheat flour was used, the AA content increased by 65, 76 and 89% for biscuits baked at 180°C for 7, 10 and 13 min, respectively. Similar results were obtained by Mesías *et al.* (2019) who showed that wholegrain biscuits had an AA content higher with nearly 50% compared to refined cereal biscuits.

By analysing 71 different cereal-based products, Nematollahi *et al.* (2019) concluded that the AA level is dependent on the type of raw material and ingredients used, formulation recipe and baking parameters. The results showed that the AA content ranged between 76.68 to 583.60 µg/kg, with the highest average amount found for wafer (233.94 µg/kg).

Very little information about the AA level of Romanian commercialized food and the population exposure to this contaminant is known. Taking this into consideration, the aim of this paper was to realize an overview of AA levels in cereal-based products commercialized on the Romania market between the years 2018 and 2019. These products, due to their relatively widespread consumption, could be a key source of AA in the Romanian diet.

Materials and methods

Food samples

A total of 46 samples of cereal-based products found on the Romanian market from which 19 samples are in the bread category, 13 samples in the biscuits and wafers group, 6 samples in the crackers category and 8 samples are products similar to those mentioned were analysed for the AA content. These food products were analysed between the years 2018 and 2019, 35 samples being analysed in 2018, and 11 samples in 2019.

The soft bread samples were sliced, cut into small pieces and then dried at a temperature of 50°C, for 3 hours in a drying and heating chamber (Binder GmbH, Germany). Before and after drying the bread, the moisture content was determined using a moisture analyzer (Mettler Toledo, China). The samples of dried bread, biscuits with and without cream and other similar products were milled and homogenized in a laboratory mixer (Büchi B-400, BÜCHI Labortechnik AG, Switzerland). From the milled and homogenized samples, 3 g of bread sample and 1.5 g of biscuits and other similar products samples, respectively was weighed using an analytical balance to the nearest 0.1 mg (Precisa Gravimetrics AG, Switzerland). The samples were subjected to analysis for the determination of acrylamide content by GC-MS/MS.

Reagents and reference standards

Native AA of concentration 1004 µg/mL in methanol (99% purity) was purchased from Restek (Benner Circle, Bellefonte, U.S.) and labelled acrylamide (1,2,3-¹³C

labelled AA) (99% purity) of concentration 1000 µg/mL in methanol (+100 ppm hydroquinone) used as internal standard (IS) was acquired from Cambridge Isotope Laboratories (Andover, MA, USA). All solvents and reagents were of analytical grade, special for chromatography and were purchased from Merck (Darmstadt, Germany), LGC Promochem GmbH (Wesel, Germany), Alfa Aesar (Thermo Fisher Scientific, USA). Ultrapure water was obtained through a PURELAB Option-S7 and PURELAB Ultra Ionic system (Elga Labwater, High Wycombe, UK).

To assess the quality control, a certified reference material “acrylamide in rusk” (ERM[®]-BD274, BAM, Berlin, Germany) with the certified value of 74 µg/kg was used.

Preparation of reference solutions

Stock solutions of AA and IS (100 mg/L) were prepared in ultrapure water in amber vials. Working solutions of AA of concentration 10, 1 and 0.1 mg/L, respectively, and working solution of IS of concentration 10 mg/L were prepared from the stock solutions by dissolving in ultrapure water.

Preparation of calibration solutions

The calibration solutions were prepared according to the procedure described in a previous study (Negoiță and Culețu, 2016). Two calibration curves were realized in the range of 0.025– 5 mg/L based on the derivatized standard solutions and plotting the ratio of the peak area of 2-bromopropenamide (2-BPA) and 2-BP(¹³C)A against the concentration of 2-BPA.

Acrylamide determination

Acrylamide analyses were realized on a gas chromatograph, type TRACE GC Ultra, coupled with triple quadrupole mass spectrometer (TSQ Quantum XLS) from Thermo Fisher Scientific (USA). A capillary column based on polyethylene-glycol, TraceGOLD™ TG-WaxMS (30 m x 0.25 mm i.d. x 0.25 µm, Thermo Fisher Scientific, USA) was used as stationary phase. The mobile phase was helium.

The analyses were performed in the electron impact ionization operation mode, positive (⁺EI); acquisition mode: selected reaction monitoring (SRM) and ion scanning mode - Product ("Product").

AA analysis was performed according to the method described by Negoiță and Culețu (2016). Briefly, the following steps were followed: samples were milled and homogenized in a Büchi professional mixer, AA was extracted in water and IS was added. Samples were treated with Carrez solutions for deproteinization, and then it was centrifugated. For derivatization the bromine compounds were used and the derivatized compound 2,3-dibromopropanamide (2,3-DBPA) obtained was extracted in a mixture of ethyl acetate: hexane and after that it was concentrated in 2 steps, first with a vacuum evaporation system (40°C, 156 – 90 mBar), and then to dryness under a nitrogen stream. The clean-up was realized on a florisil column, and the 2,3-DBPA derivative was eluted with acetone, concentrated till dryness and

the residue was redissolved in ethyl acetate and triethylamine. The final extract of 2-BPA was injected to GC-MS/MS.

AA quantification was performed by the internal standard method.

Method performance

The method used for acrylamide quantification in bread, biscuits and other bakery products was described by Negoită and Culețu (2016). The recovery of the procedure was in the range of 97% - 105%. The relative standard deviations for precision, repeatability and reproducibility were 0.4– 4.9% and 0.7– 5.8%, respectively.

The Commission Regulation (EU) 2017/2158 states that the method used for the analysis of AA must comply with the following criteria: LOQ less than or equal to two fifths of the benchmark level (for benchmark level < 125 µg/kg) and less ≤ 50 µg/kg (for benchmark level ≥ 125 µg/kg), while LOD ≤ 3/10 of LOQ (Tabel 1). The analytical method used was characterized by a good sensitivity, with a LOD of 1.23 µg/kg, and a LOQ of 3.7 µg/kg for cereal-based products, clearly meeting the required conditions.

According to the same Regulation, the benchmark level for the presence of AA in cereal-based food products are presented in Table 1.

Table 1. Benchmark levels for the presence of acrylamide in cereal-based products and method sensitivity set by Commission Regulation (EU) 2017/2158.

Foodstuffs	BL* (µg/kg)	Sensitivity	
		LOD (µg/kg)	LOQ (µg/kg)
Soft bread			
- Wheat based bread	50		
- Soft bread other than wheat-based bread	100	≤ 3/10*LOQ	≤ 2/5*BL
Biscuits and wafers	350		
Crackers with the exception of potato-based crackers	400		
Products similar to the other products in this category (fine bakery products)	300	≤ 3/10*LOQ	≤ 50

*Acrylamide benchmark levels (BL)

The accuracy of the results was demonstrated for cereal-based matrix (biscuit-cookie) by participation to two proficiency tests (PT) launched by the Food Analysis Performance Assessment Scheme (FAPAS) program, yielding z-scores of -0.2 (PT 3084, 2018) and -0.1 (PT 3094, 2019), respectively. All analyses were performed in duplicate and results were expressed as µg/kg AA.

Results and discussion

In order to determine the AA content of cereal-based products, two calibration curves were realized by the method of least squares in the ranges 0.025– 0.5 mg/L (Figure 1) and 0.4– 5 mg/L (Figure 2) choosing the internal standard method. The

correlation was assessed to be linear for a value greater than 0.99 for the correlation coefficient (R).

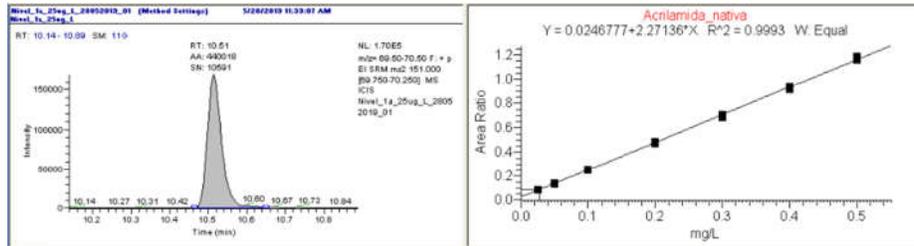


Figure 1. Calibration curve (0.025 - 0.5 mg/L) for acrylamide (2-BPA) detection analysis and acrylamide peak

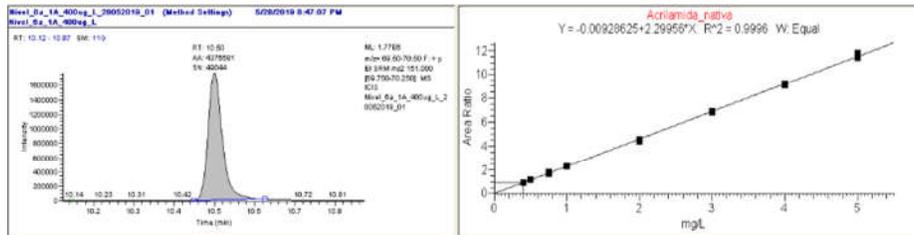


Figure 2. Calibration curve (0.4 - 5 mg/L) for acrylamide (2-BPA) detection analysis and acrylamide peak

Quality control (QC) in the laboratory (Figure 3) was performed with each set of samples analyzed using a certified reference material “acrylamide in rusk”, and the bias obtained was 0.58% (n= 33).

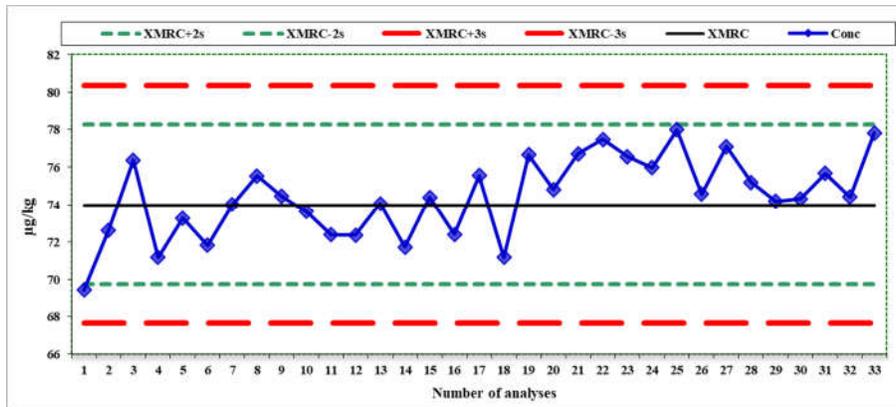


Figure 3. Quality control chart for the determination of acrylamide in the QC samples

The results obtained by analyzing the samples showed that the AA content between the same category of food and between groups varied. It is well known that the AA

formation depends on the asparagine and reducing sugars content of the raw materials, as well as the processing conditions.

AA content of soft bread

Bread is one of the most consumed cereal-based products in Romanian diet, Romania being one of the top three bread consuming countries in Europe (FRD, 2016).

A total of 19 commercial bread samples of which 14 samples were from the wheat-based bread category, and 5 samples were from the soft bread other than wheat-based bread group were analysed for the AA content (Table 2).

In the bread samples obtained from wheat, the AA content varied between 5.63 and 45.01 $\mu\text{g}/\text{kg}$, while for bread obtained from other cereals the AA content varied between 21.78 and 85.05 $\mu\text{g}/\text{kg}$.

Table 2. Acrylamide content of bread samples analysed.

No.	Bread	AA content, $\mu\text{g}/\text{kg}^*$
Wheat-based bread		
1	Classic toast	8.53 \pm 0.44
2	Mini bread baguette with sesame	19.29 \pm 1.18
3	Whole toast	8.28 \pm 0.69
4	Hot-dog roll	12.24 \pm 0.44
5	Wheat bun	28.08 \pm 0.26
6	Peasant bread	9.79 \pm 0.05
7	Sliced Classic white loaf bread	5.63 \pm 0.22
8	Wheat bread	7.11 \pm 0.32
9	Graham toast	16.91 \pm 0.62
10	Sliced wheat bread	9.34 \pm 0.98
11	Wheat bread	6.79 \pm 0.15
12	Lebanese bread 1	45.01 \pm 2.04
13	Lebanese bread 2	30.94 \pm 1.56
14	Lebanese bread 3	31.54 \pm 0.27
Soft bread other than wheat-based bread		
1	Bread made of rye MIX	36.56 \pm 0.11
2	Bread made with rye (rye -potato)	35.86 \pm 0.17
3	Rye bread	85.05 \pm 0.10
4	Bread with fibre and seeds	74.17 \pm 0.49
5	Rye bread toast	21.78 \pm 1.49

*Data are expressed as mean values \pm standard deviations (SDs) of two replicates

Three samples of Lebanese bread from the same brand were analysed between 2018 and 2019, of which one was analysed in 2018 and presented the highest level of AA from the wheat bread category (45.01 $\mu\text{g}/\text{kg}$), and the other two were analysed in 2019. As it can be noticed from Table 2, the AA content of this product

decreased in 2019 with about 30% as a result of the mitigation measures applied by the food business operator in order to reduce AA formation in this product.

From all of the soft bread analysed, the highest AA content was obtained for rye-bread. The type of cereal used in the formulation recipe of bread is a factor which influences the AA level. In some bread model systems realized by Capuano *et al.* (2009) it was demonstrated that the flour type has a determinant effect on the AA content, higher levels being obtained when in the recipe rye flour is used, followed by whole wheat and wheat flours. Similar results were obtained by Przygodzka *et al.* (2015) who showed that the cereal from which the flour is obtained has implication on the AA level of bread, the highest level being found in rye bread compared to wheat and spelt breads.

Forstova *et al.* (2014) studied the AA content of bread produced in various Czech bakeries and, as in our study, the level was low, ranging between 7 and 22 µg/kg. The content of AA detected in the bakery products analysed in Romania were similar with the ones measure in Lesser Poland, where the level was between undetectable to 77.7 µg/kg, with an average of 29.74 µg/kg for the 15 products analysed (Cieślík *et al.*, 2019). In a survey carried out in Iran, the AA values of traditional and industrial bread samples were less than 83.3 µg/kg (Eslamizad *et al.*, 2019).

The AA levels for all bread samples analysed were below the benchmark levels presented in the Commission Regulation (EU) 2017/2158.

Soft bread contributes to AA exposure to adults in a percent of 23%, followed by biscuits, crackers and crisp bread (EFSA, 2015). Adult chronic dietary exposure to AA estimated by EFSA was between 0.4 and 0.9 µg/kg b.w./day. Exposure to AA from bread in the Romanian population was estimated considering total data per capita consumption of this product of 95 kg/capita/year as indicated by FRD (2016). Taking into account that in Romania, the bread intake for an adult weighing 80 kg is 260 g/day (FRD, 2016), and based on the average level of AA from bread obtained in this study (average of 25.99 µg/kg, Table 2), it can be concluded that bread contributes to a chronic dietary exposure of 0.9 µg/kg b.w./day with about 9.37%. In Poland, similar as in Romania, bakery products are very consumed and they are the main source of AA. In the study realized by Cieślík *et al.* (2019) the mean AA exposure from the consumption of bread ranged between 0.01 and 0.05 µg/kg b.w./day, the AA exposure decreasing with increasing age. When evaluating the dietary exposure to AA of Slovenian adult population, Mencic *et al.* (2020) calculated an average dietary exposure to AA of toast and bread products of 0.21 µg/kg b.w./day, these foodstuffs contributing with 56% to dietary exposure to AA. In France, the estimated dietary exposure to AA among children and adults was 0.020, and 0.024 µg/kg b.w./day, respectively when bread and bread products were consumed (Sirot *et al.*, 2012).

AA content of biscuits and wafers

Biscuits are a cereal-based food product which include sugars and fats in their composition. Sugar is one of the main ingredients in the recipe and at high

temperatures it participates to the Maillard reaction, thus influencing the AA content of the end product. The difference in biscuits composition and in the processing conditions applied can easily explain the observed variability between samples. Studies showed that AA formation is dependent on the type and concentration of sugars and revealed that fructose contribute to the formation of a higher level of AA compared to glucose (Mustăţea *et al.*, 2015, Nguyen *et al.*, 2016, Nguyen *et al.*, 2017). Also, the type of fat and wheat flour used influences the AA level of biscuits (Negoiţă *et al.*, 2017b).

On Romanian market, the most consumed biscuits are cream “sandwich” biscuits, followed by simple biscuits (FRD, 2016). Due to the high rate of consumption, biscuits are an important source of AA intake. Out of the biscuits and wafers category, a total of 13 samples were analysed to determine the AA content (Table 3).

Table 3 Acrylamide content of biscuits and wafers samples analysed.

No.	Biscuits and wafers	AA content, µg/kg*
1	Double-biscuits with cacao cream	548.80 ± 16.53
2	Mini-biscuits with cacao flavour	278.53 ± 0.46
3	Cacao biscuits with cacao cream	207.23 ± 0.14
4	Mini-cacao biscuits with cacao cream	275.33 ± 2.73
5	Cacao biscuits with cacao cream	255.83 ± 3.47
6	Mini-cacao biscuits with vanilla flavoured cream	165.30 ± 1.29
7	Biscuits with honey	304.87 ± 7.93
8	Mini-biscuits with cacao cream	426.03 ± 2.43
9	Sweet biscuits 1	538.26 ± 15.90
10	Sweet biscuits 2	538.95 ± 4.22
11	Sweet biscuits 3	166.97 ± 9.36
12	Cookies	8.55 ± 0.04
13	Éclair shells	29.45 ± 2.32

*Data are expressed as mean values ± standard deviations (SDs) of two replicates

For this group of cereal-based products, 4 samples analysed between 2018 and 2019 exceeded the benchmark level of 350 µg/kg established by Regulation 2017/2158. The highest AA content was recorded for double-biscuits with cacao cream (548.80 µg/kg), followed by 2 samples of sweet biscuits (1- 538.26 µg/kg and 2- 538.95 µg/kg) and mini-biscuits with cacao cream (426.03 µg/kg). The sweet biscuits (1 and 2) for which it was determined a higher AA content than the one set out for Commission Regulation (EU) 2017/2158 were analysed in 2018. It can be noticed that for this sample analysed in 2019 the AA content decreased by about 70% as a result of the mitigation measures applied by food business operators.

From the wafers group, cookies and éclair shells were analysed and an AA level less than 30 µg/kg was detected.

From the biscuits and wafers category analysed, 31% of the tested samples exceeded the benchmark level of Regulation 2158/2017. Michalak *et al.* (2019) investigated the AA level of food products purchased from the Poland supermarkets and in the case of biscuits, the AA content was exceeded for all ten samples analysed, the mean AA content being higher with 49% than the benchmark level. In a study realized by Tölgyesi and Sharma (2020) on 42 biscuits samples purchased from Hungary shops it was shown that the AA level ranged between 20 and 342 $\mu\text{g}/\text{kg}$, being less than the one set by legislation. When comparing the AA content of Spanish biscuits marked on 2007 versus 2019, it was noticed that an important decline of 45% was obtained for wheat-based biscuits as a result of the mitigation strategies applied by the biscuit manufacturers (Mesías *et al.*, 2019). To estimate the exposure to AA from biscuits intake in the Romanian population was taken into account the amount of 2.1 kg/capita/year as indicated by FRD (2016). Considering that in Romania, the biscuits and similar products intake for an adult weighing 80 kg is 6.03 g/day (FRD, 2016) and based on the average level of AA obtained in this study for the biscuits (average of 288.01 $\mu\text{g}/\text{kg}$, Table 3), it can be concluded that biscuits contributes to a chronic dietary exposure of 0.9 $\mu\text{g}/\text{kg}$ b.w./day with about 2.40%. In a study realized in Slovenia, the dietary exposure to AA by consumption of biscuits and wafers ranged between 0.03 and 0.04 $\mu\text{g}/\text{kg}$ b.w./day, these products contributing with 9% of the dietary exposure to AA of adult population (Mencic *et al.*, 2020). In France, the estimated dietary exposure to AA by consumption of sweet biscuits was 0.017 $\mu\text{g}/\text{kg}$ b.w./day for adults, while for children was 0.075 $\mu\text{g}/\text{kg}$ b.w./day (Sirot *et al.*, 2012).

AA content of crackers

In Romania, from the salty snacks category, the most consumed products are pretzels, followed by crackers and a less percent of sticks, mixes and salty sticks (FRD, 2016).

A total of 6 samples from the crackers category were analysed for AA content determination (Table 4). The benchmark level for this category is set to 400 $\mu\text{g}/\text{kg}$ (Regulation 2017/2158) and none of the analysed samples did not exceeded this level, the AA content varying between 13.92 and 167.58 $\mu\text{g}/\text{kg}$. It is known that the difference in the food composition and in the processing parameters applied influence the AA content, the highest values being found in crackers in which just salt was added, compared to the ones where another ingredient was added.

Table 4 Acrylamide content of crackers samples analysed.

No.	Crackers	AA content, $\mu\text{g}/\text{kg}$ *
1	Rusk	13.92 \pm 0.43
2	Pretzels with white wine	73.84 \pm 3.86
3	Pretzels with salt and cumin	59.69 \pm 2.99
4	Pretzels with salt and poppy seeds	81.00 \pm 1.42
5	Pretzels with salt	167.58 \pm 2.24
6	Mini brezel salt	111.73 \pm 2.03

*Data are expressed as mean values \pm standard deviations (SDs) of two replicates

Similar results were obtained by Cieřlik *et al.* (2019) and Nematollahi *et al.* (2019) who found for pretzels an AA content ranging from 69.3 to 124 µg/kg, and 126.90 µg/kg, respectively. Higher AA levels were found in the survey conducted by Gündüz *et al.* (2017) where the mean AA content for crackers was 604 µg/kg.

AA content of fine bakery products

The AA level of fine bakery products was evaluated as well on 8 samples. As it can be noticed from Table 5, none of the analysed samples from this category did not exceed the benchmark level of 300 µg/kg set out by European Commission 2017/2158. In the fine bakery composition, the addition of sugar or similar ingredients can affect the AA level of end product. For sponge cake an AA level of 16.67 µg/kg was found in our study, while Nematollahi *et al.* (2019) found for the same product commercialized on Iran market an average content of 148.33 µg/kg.

Table 5 Acrylamide content of fine bakery samples analysed.

No.	Fine bakery	AA content, µg/kg*
1	White cake	12.93 ± 0.47
2	Rum baba	12.57 ± 0.11
3	Amandine	15.02 ± 0.94
4	Sponge cake	16.67 ± 0.53
5	Cremschnitte	99.01 ± 6.72
6	Marble cake without fruit	30.96 ± 1.32
7	Marble cake	29.45 ± 2.32
8	Doughnuts	27.87 ± 0.20

*Data are expressed as mean values ± standard deviations (SDs) of two replicates

In this category of food products, the highest AA content was obtained in cremeschnitte sample (99.01 µg/kg), the other products analysed having a level ranging between 12.57 and 30.96 µg/kg. In a survey realized by Razia *et al.* (2016), similar results were obtained for cakes, the AA level being less than 35 µg/kg.

Conclusions

AA was present in all samples analyzed, this indicating that cereal-based products contribute to AA intake as are daily consumed. From the total of 46 samples, AA was detected in all cereal-based products analyzed between 2018 and 2019 at levels ranging from 5.63 µg/kg to 548.80 µg/kg.

Of all the analyzed samples (46), only 8.7% (4) exceeded the level of AA established by Regulation (EU) 2017/2158. The highest AA content was found in biscuits, like this contributing to AA exposure.

Mitigation strategies should be applied to reduce the AA formation in all cereal based-products, not only in biscuits, thus reducing the AA exposure among the population. It is important to have a balanced healthy diet in which the consumption of bakery products to be limited.

Acknowledgments

This study was achieved through Core Programme (PN 19 02), with the support of the Ministry of Research and Innovation (MCI), contract 22N/2019, project PN 19 02 03 01.

References

- AL Karim, S., El Assouli, S., Ali, S., Ayuob, N., El Assouli, Z. 2015. Effects of low dose acrylamide on the rat reproductive organs structure, fertility and gene integrity. *Asian Pacific Journal of Reproduction*, **4**(3), 179-187.
- Capuano, E., Ferrigno, A., Acampa, I., Serpen, A., Açar, Ö., Gökmen, V., Fogliano, V. 2009. Effect of flour type on Maillard reaction and acrylamide formation during toasting of bread crisp model systems and mitigation strategies. *Food Research International*, **42**, 1295-1302.
- Cieślak, I., Cieslik, E., Topolska, K., Surma, M. 2019. Dietary acrylamide exposure from traditional food products in Lesser Poland and associated risk assessment. *Annals of Agricultural and Environmental Medicine: AAEM*, **27**(2), 225-230.
- Claus, A., Mongili, M., Weisz, G., Schieber, A., Carle, R., 2008. Impact of formulation and technological factors on the acrylamide content of wheat bread and bread rolls. *Journal of Cereal Science*, **47**, 546-554.
- Eslamizad, S., Kobarfard, F., Tsatsakou, C., Tsatsakis, A., Tabib, K., Yazdanpanah, H. 2019. Health risk assessment of acrylamide in bread in Iran using LC-MS/MS. *Food and Chemical Toxicology*, **126**, 162-168.
- European Commission (EU). 2017. Commission Regulation 2017/2158 of 20 November 2017 establishing mitigation measures and benchmark levels for the reduction of the presence of acrylamide in food. *Official Journal of the European Union*, L304, 24-44.
- European Food Safety Authority (EFSA). 2015. Scientific opinion on acrylamide in food. EFSA Panel on contaminants in the food chain (CONTAM). *EFSA Journal*, **13**(6), 4104.
- Food Drink Europe. 2019. Acrylamide toolbox. Available from: https://www.fooddrinkeurope.eu/uploads/publications_documents/FoodDrinkEurope_Acrylamide_Toolbox_2019.pdf.
- FRD. 2016. Food market in Romania. A FRD Center publication for the Embassy of the Kingdom of the Netherlands in Romania, available from: <https://www.dutchromaniannetwork.nl/wp-content/uploads/2017/01/Food-Report-Romania-2016.pdf>.
- Forstova, V., Belkova, B., Riddellova, K., Vaclavik, L., Prihoda, J., Hajslova, J. 2014. Acrylamide formation in traditional Czech leavened wheat-rye breads and wheat rolls, *Food Control*, **38**, 221-226.
- Gündüz, C.P.B., Bilgin, A.K., Cengiz, M.F. 2017. Acrylamide contents of some commercial crackers, biscuits and baby biscuits. *Akademik Gıda*, **15**(1), 1-7.
- International Agency for Research on Cancer (IARC). 1994. *Monographs on the Evaluation of Carcinogenic Risks of Chemicals to Humans*, **60**, 389, Lyon, France.
- FAO/WHO. 2011. Evaluation of certain contaminants in food: Seventy-second report of the Joint FAO/WHO Expert Committee on Food Additives, Rome.

- Kacar, S., Sahinturk, V., Mehtap, K. 2019. Effect of acrylamide on BEAS-2B normal human lung cells: Cytotoxic, oxidative, apoptotic and morphometric analysis. *Acta Histochemica*, **121**, 595-603.
- Matoso, V., Bargi-Souza, P., Ivanski, F., Romano, M.A., Romano, R.M. 2019. Acrylamide: A review about its toxic effects in the light of developmental origin of health and disease (DOHaD) concept. *Food Chemistry*, **283**, 422-430.
- Mencin, M., Abramovič, H., Vidrih, R., Schreiner, M. 2020. Acrylamide levels in food products on the Slovenian market, *Food Control*, 107267.
- Mesías, M., Morales, F.J., Delgado-Andrade, C. 2019. Acrylamide in biscuits commercialized in Spain: a view of the Spanish market from 2007 to 2019. *Food & Function*, **10**, 6624-6632.
- Michalak, J., Czarnowska-Kujawska, M., Gujska, E. 2019. Acrylamide and thermal-processing indexes in market-purchased food. *International Journal of Environmental Research and Public Health*, **16**, 4724, 1-9.
- Mustăţea, G., Popa, M.E., Negoită, M., Israel Roming, F. 2015. Asparagine and sweeteners – how they influence acrylamide formation in wheat flour biscuits? *Abstracts/Journal of Biotechnology*, **208**, S5-S120.
- Negoită, M., Culeţu, A., 2016. Application of an accurate and validated method for identification and quantification of acrylamide in bread, biscuits and other bakery products using GC-MS/MS system. *Journal of Brazilian Chemical Society*, **27**(10), 1782-1791.
- Negoită, M., Mihai, A.L., Iorga, E., 2017a. Influence of technological factors on acrylamide level from biscuits. *Scientific Bulletin. Series F. Biotechnologies*, **XXI**, 149-158.
- Negoită, M., Iorga, E., Mihai, A.L., Spadaro, G., 2017b. Investigation regarding the influence of certain types of fat content on acrylamide level in biscuits. *Journal of Hygienic Engineering and Design*, **15**, 31-41.
- Nematollahi, A., Kamankesh, M., Hosseini, H., Ghasemi, J., Hosseini-Esfahani, F., Mohammadi, A. 2019. Investigation and determination of acrylamide in the main group of cereal products using advanced microextraction method coupled with gas chromatography-mass spectrometry. *Journal of Cereal Science*, **87**, 157-164.
- Nguyen, Ha T., Van der Fels-Klerx, H.J. (Ine)., Peters, R.J.B., Van Boekel, M.A.J.S. 2016. Acrylamide and 5-hydroxymethylfurfural formation during biscuit baking: Part I: Effect of sugar type, *Food Chemistry*, **192**, 575-585.
- Nguyen, Ha T., Van der Fels-Klerx, H.J. (Ine)., Van Boekel, M.A.J.S. 2017. Acrylamide and 5-hydroxymethylfurfural formation during biscuit baking. Part II: Effect of the ratio of reducing sugars and asparagine, *Food Chemistry*, **230**, 14-23.
- Przygodzka, M., Piskula, M.K., Kukurová, K., Ciesarová, Z., Bednarikova, A., Zieliński, H. 2015. Factors influencing acrylamide formation in rye, wheat and spelt breads, *Journal of Cereal Science*, **65**, 96-102.
- Razia, S., Bertrand, M., Klaus, V., Meinolf, G.L. 2016. Investigation of acrylamide levels in branded biscuits, cakes and potato chips commonly consumed in Pakistan. *International Food Research Journal*, **23**(5), 2187-2192.
- Sirost, V., Hommet F., Tard, A., Leblanc, J.-C. 2012. Dietary acrylamide exposure of the French population: Results of the second French Total Diet Study, *Food and Chemical Toxicology*, **50**, 889-894.

-
- Tölgyesi, Á., Sharm, V.K. 2020. Determination of acrylamide in gingerbread and other food samples by HILIC-MS/MS: A dilute-and-shoot method. *Journal of Chromatography B*, **1136**, 121933.
- Žilić, S., Aktağ, I.G., Dodig, D., Filipović, M., Gökmen, V., 2020. Acrylamide formation in biscuits made of different wholegrain flours depending on their free asparagine content and baking conditions. *Food Research International*, **132**, 109109