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Evaluation of acrylamide levels in cereal products from the Romanian market during the 2017 and 2018 period

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Abstract

Cereal products are the most consumed in Romania being the main contributors to daily acrylamide exposure. The paper aims to present for the first time a general situation regarding the evolution of the acrylamide levels content from cereal products, on the Romanian market, during 2017-2018 periods, as a result of legislative measures imposed by the European Union (EU). For this purpose, the levels of acrylamide in 55 selected cereal products samples were evaluated. The cereal products analyzed were grouped in biscuits, confectionery, expanded cereals, bakery products and specialties. The acrylamide content from the cereal products were detected using GC-MS/MS method. The highest level of acrylamide was found in biscuits, whereas the lowest level was determined in bakery products. The most of the cereal products samples analyzed (90.9%) was below the reference levels established by the EU Regulation for the acrylamide level from 2017 EC (2013/647/EU) and 2018 EC (2017/2158/EU). From the 55 cereal products analyzed, only 5 biscuits samples exceeded the reference levels established by the European Commission, one in 2017 and four in 2018 period.

Keywords: acrylamide; exposure; risk assessment; public health

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Introduction

In many countries, cereal products are known as important basic foods which contain essential nutrients such as proteins, carbohydrates, fibers and vitamins (1). Beside these nutrients, cereal products may contain compounds formed during heat treatment, such as acrylamide (AA), hydroxymethylfurfural and their derivatives (2, 3). Assessment of the presence and reducing the AA levels formed in cereal foods are a great concern in many countries (4).

The International Agency for Research on Cancer (IARC 1994) classified acrylamide as "potentially carcinogenic to humans", and in 2001, the Scientific Committee on Toxicity, Ecotoxicity and the Environment demonstrated its toxic properties: cytotoxic and mutagenic effects in people and animals; exhibits neurotoxic and genotoxic effects to both somatic and germ cells (causing cell damage at a genetic level); causes infertility, eye infection, weakness and irritability; causes paralysis of the cerebrospinal system (2, 4, 5, 6, 7, 8, 9). The Expert Panel on Contaminants in the Food Chain stated that the margins of exposure for acrylamide indicate a concern for neoplastic effects based on animal tests (EFSA, 2015) (10). AA is transferred through the blood stream to all parts of the human body. It may be found in many parts of the human body such as kidney, brain, heart, liver and even breast milk (8). Acrylamide is a non-volatile and a low molecular weight compound with high solubility in water, it is very reactive in air and it is rapidly polymerized (4,7). Acrylamide is formed in foodstuffs if the thermal processing such as heating or frying in an oven or in a frying pan or by microwave heating, while no acrylamide has been detected in boiled food products (2, 4). The main pathway leading to acrylamide in

foodstuffs is the Maillard reaction, a reaction between a carbonyl source such as glucose and fructose and proteins/amino acids (mainly asparagine), but acrylamide can also be formed in other ways, including decarboxylation and deamination of asparagine, from lipid degradation, dehydration or decarboxylation of organic acids, from ammonia released through thermal degradation of amino acids and proteins (1, 3, 4, 10). During the baking of cereal products, a series of nonenzymatic reactions take place between carbohydrates and proteins that are responsible for the development of the desirable flavor and the brown color of heated foods (10, 11,12, 13).

The level of acrylamide in different foodstuffs is between a few parts per billion (ppb) and may exceed the value of 1000 ppb (14). The tolerable daily intake of acrylamide through diet in adults is about 0.3-0.6 µg per kg of body weight (bw) and, in children and adolescents, it is 0.4-0.6 µg per kg of body weight (7, 8). The expert committee evaluation of FAO/ WHO, has established that 1 µg/ kg bw /day is considered an average exposure to acrylamide, while 4 µg kg /bw / day is considered as high exposure to acrylamide (15).

The most important acrylamide dietary sources are potato derivates (French fries, crisps), cereal products (cereal-based snacks, breakfast cereals, popcorn), bread, biscuits, coffee, chocolate and olives (1, 6, 7, 10, 12, 16). Its presence was also reported in tobacco smoke (17).

In Europe, data on the presence of AA in foodstuffs has been collected since 2003 by the European Commission Joint Research Centre's Institute for Reference Materials and Measurements and since 2007 by the European Food Safety Authority (EFSA) which presented a series of reports about the collected data. Throughout the time, based on the data, the European Commission (EC) made a series of recommendations for the Member States of the European Union (EU) such as 2007/331/EC (European Commission, 2007), 2010/307/EU (European Commission, 2010), 2013/647/EU (European Commission, 2013), 2017/2151/EU (European Commission, 2017) on the reference values for AA levels in different types of foodstuffs (18).

Being a Member State of the European Union, Romania must also report data to the EFSA. Unfortunately, the amount of collected informations on AA levels found in different foodstuffs on the Romanian market is very small. From the onset of the acrylamide issue, the National Sanitary Veterinary and Food Safety Authority (NSVFSA), must monitor the acrylamide content from different foodstuffs on the market. Moreover, in Romania, within the National Research & Development Institute for Food Bioresources (IBA Bucharest), researches on the influence of the extraction ratio of wheat flour on acrylamide levels in bread was conducted between 2012-2015. The aim of this work was to evaluate the most recent data available on the acrylamide levels in the cereal products on the Romanian market in 2017 and 2018 period. This data allows us to evaluate the risks related to the exposure of the population of Romania to the acrylamide by cereal products consumption.

Materials and Methods

Samples

A total of 55 samples of bakery products, namely 22 bakery products, 8 confectionery products, 8 cereal products, 16 biscuits products, 1 specialty product (bagels) were collected from the Romanian market. The samples were analyzed separately in 2017 and 2018 period, namely 7 samples in 2017 and 48 samples in 2018 period. The samples from 2017 were analyzed only by the IBA Bucharest, whereas the samples from 2018 were analyzed by the IBA Bucharest and by the National Sanitary Veterinary and Food Safety Authority (NSVFSA).

Acrylamide analysis

Acrylamide(AA) levels from cereal products were detected only by IBA Bucharest analyzed samples. The method used by IBA Bucharest was gas chromatography coupled with mass spectrometry (GC-MS/MS) with the use of internal standard (15). The method was accredited by RENAR in 2016. 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"). The method used was previously published without any modifications including the reagents, consumables, equipment, sample preparation, method validation, acrylamide determination (19).

Acrylamide (AA) levels from cereal products were also analyzed by NSVFSA using high performance liquid chromatography coupled with a diode array detector (HPLC-UV) according to the method previously described by (20). However, the NSVFSA did not detect any AA level in their analyzed cereal products samples.

The LOQ values for the IBA and the NSVFSA methods were under 30 µg/kg which comply with the level established by the European Commission recommendations for acrylamide content in foodstuffs (2010/307/EU European Commission, 2010) (18).

Statistical analysis

The experimental data were statistically evaluated using SPSS Windows version 16.0 software (trial version, SPSS, Chicago, IL, USA). Significant differences for the data on acrylamide levels were evaluated by one-way analysis of variance (ANOVA) at significance level of 0.05.

Results

Validation of the Analytical Method used to detect AA levels in cereal products

Prior to the analysis of acrylamide, the GC-MS-MS method was validated to ensure the quality of the data. The linearity of the calibration curve was checked by a series of standard solutions of acrylamide ranged 7.23 – 312.88 µg/kg for bread and similar and 17.59 – 2191.41 µg/kg for biscuits and similar rations. The recoveries and the relative standard deviation were studied using prepared samples spiked with different concentrations of acrylamide at different concentrations: 40, 75, 80, 150, 225, 300 µg/kg for bread and other similar products and

Table 1. The linear range, limit of detection, recovery and precision of the method for acrylamide determination in bread and similar, biscuits and similar, respectively

Product	Linear range	R^2	LOD (µg/kg)	LOQ (µg/kg)	Recovery (%)
Bread and similar	7.23 – 312.88 µg/kg	0.998	2.41	7.23	93.68 – 102.93
Biscuits and similar	17.59 – 2191.41 µg/kg	0.999	4.63	13.89	93.68 – 102.93

R^2 – coefficient of regression, LOD – limit of detection, LOQ-limit of quantification

Table 2. Analysis of variance of acrylamide level in cereal products on the Romanian market on 2017 and 2018 period

Parameter	Year		F value
	2017	2018	
Acrylamide (µg/kg)	355.4 (10-538.1)[a]	91.03 (9-509.5)[a]	0.2^{ns}

ns, non-significantly ($p > 0.05$), a – statistical group

250, 500, 750, 1000, 2000 µg/kg for biscuits and other similar products. The results can be seen in **Table 1**. The method showed a good linearity in concentrations ranging from 7.23 – 312.88 µg/kg for bread and similar and 17.59 – 2191.41 µg/kg for biscuits and similar.

Discussion

In Romania, at a national level, there are not many food samples analyzed in order to determine the acrylamide content found in them. The data obtained for the AA levels in the cereal products on the Romanian market between 2017 and 2018 period are shown in **Table 2**. It is quite difficult to compare the AA levels from this period because cereal products are heterogeneous. In addition, very few samples were analyzed in 2017 compared to 2018 period. The category of cereal products in 2017 included especially biscuits, whereas in 2018, the most samples analyzed were different types of bread from wheat flour.

This data is reported only by IBA-Bucharest which detected AA in cereal products. The NSVFSA did not detect any AA levels in the few cereal products samples they analyzed in 2018 period. Moreover, in 2017, the NSVFSA, which is the Romanian National Authority for food safety, has not made any analyses to determine the acrylamide content from foodstuffs present on the market. However, in 2017, the IBA-Bucharest analyzed 7 bakery products samples on the Romanian market, namely 5 samples of various types of biscuits, 1 bread sample and 1 donut sample. These were the only cereal products samples analyzed for the AA level on the Romanian market in 2017 and the assessment was made by a research institute and not by a national authority. In 2018 the number of cereal products samples analyzed in Romania at a national level was higher, that is 48, probably as a result of the reference adopted by the Commission Regulation (EU) 2017/2158/EU (18).

The interest of the authorities and operators in the food industry regarding the monitoring of acrylamide content in foodstuffs was still very low. The NSVFSA analyzed only 14 bakery products on the Romanian market, and the rest of the samples, that is 34, were analyzed by the IBA research institute. The samples collected by the NSVFSA within the Surveillance Program were from 5 Romanian counties (Timis, Prahova, Iasi, Dolj, Constanta) and different types of units such as supermarkets, hypermarkets, food stores. These were the first samples analyzed and reported in Romania by a national authority, namely NSVFSA, according to the rules of the European Commission. Moreover, the Commission Regulation (EU) 2017/2158/EU states that the national authorities from the European Union must provide information, not only about the presence of AA in the foodstuffs which are present on the market, but they must also establish and apply measures to reduce the presence of acrylamide, as well as to respect the levels imposed by the European legislation. Furthermore, due to the fact that there is not enough data available regarding the presence of AA in foodstuffs on the European Union markets, on the 7th of November 2019, the European Commission makes again the Recommendation 2019/1888/EU to the national authorities to monitor the presence of acrylamide in certain food products from the EU and to adopt possible risk management measures in order to reduce the AA level.

In accordance with the European Recommendations, in **Figure 1** you can see that the mean values of the AA levels in the

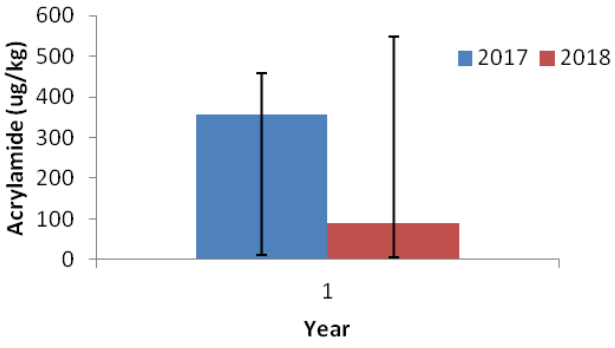

Figure 1. Evolution of acrylamide level in cereal products on the Romania market on 2017 and 2018 period. Black bar – error bar – represens the minimum and the maximum of acrylamide level.

Table 3. Analysis of variance of acrylamide level in cereal products type on the Romanian market

Parameter	Cereals products type					F value
	Biscuits	Confectionary	Bakery	Expanded cereals	Specialties	
Acrylamide (µg/kg)	330.7 (24.1-549.2)	28.3 (9.2-99.3)	27 (6.1-85.4)	54.0 (48.0-60)	188 (0)	26.26***

***$p < 0.001$

Romanian cereal products recorded a significant decrease in 2018 compared to 2017 period. These big differences between AA mean values during this period may be attributed to the type and number of cereal samples analyzed than to other technological factors. The high mean value of acrylamide in 2017 compared to 2018 is probably due to the fact that, from 7 cereal products samples analyzed in 2017, 5 were biscuits type which has, according to Commission Regulation (EU) 2017/2151/EU, higher reference level for AA compared to other cereals products type. The data we obtained complies with the existing trend at the European level. It is worth mentioning that, since 2003, when scientific reports on AA data in foodstuffs have been begin to be collected in Europe, the reference levels of the AA established by European Commission has decreased periodically. This decrease of the AA levels throughout the years indicates the effectiveness of the strategies taken to a European level by the food industry based on the control of recipe, processing factors or agronomical ones (18). Different studies over the years reported a significant decrease of the AA level in foodstuffs, including cereals. In Belgium, a comparison made between two periods, 2008-2013 versus 2002-2007 showed a decrease in the AA content from the cereal products over the years (17), whereas in Spain, the AA content from cereal products significantly decreased from 2006 to 2018 up to 60% (21).

Depending on the type of cereal analyzed, a great variation of AA content was recorded among the samples as it may be seen in **Table 3**.

The samples of biscuits contain the highest level of AA, whereas the bakery samples contain the lowest level of AA. It is well known that the main precursors of the AA formation in grain foods are reducing sugars and asparagine (Asn) through Maillard reactions (20). The AA formation is determined by sugars concentration, the type of amino acids present and technology of processing. Product formulations along with baking parameters are the most important factors affecting AA formation (22). In the baking process there are two main stages, namely the development and the drying stage. In the first stage, some physical changes in the dough take place such as protein denaturation, starch gelatinization and fat melting. In the second stage, the dehydration process occurs in a more intense way with crust forming and some chemical changes take place with the formation of melanoidins and carbonyl compounds which give the brown color to the crust and complete the aroma of the cereal products (23). During this second stage of baking, some contaminants, such as acrylamide, are formed (3). It seems that the dehydration process at high temperature favors AA formation. The biscuits and specialties reached the highest level of AA in the cereal products analyzed. This fact can be explained since both product categories are processed until a low final humidity, which means that the high dehydration process took place. Also, for biscuits type, the AA content was much higher, probably due to its sugars content from its recipe which has the ability to promote the Maillard reactions (24) and also the AA formation. Additionally, a significant decrease of the AA level was noticed in expanded cereals and confectionery products. This is the result of the fact that these products are subjected to less intense heat treatment than biscuits and specialties. Bakery products, on the other hand, presented the lowest level of AA.

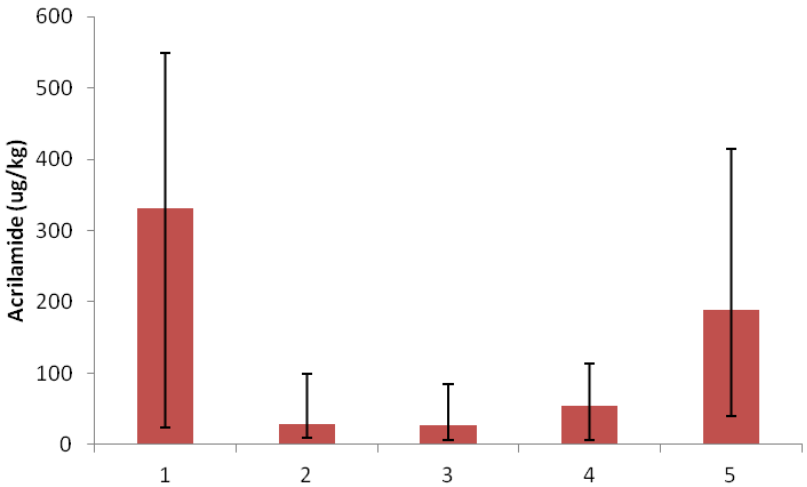

Figure 2. Evolution of acrylamide level in cereal products on the Romania market: 1 – biscuits, 2 – confectionery products, 3- bakery products, 4 – products from expanded cereals, 5 – specialities. Black bar – error bar – represens the minimum and the maximum of acrylamide level.

Table 4. Indicative values and benchmark levels for AA in cereal products set by the European Commission

Cereal products	Year	
	Indicative value 2013 (ppb)	Indicative value 2017 (ppb)
Bread made from wheat	80	50
Soft bread, other than wheat bread	150	100
Biscuits and waffles	500	350
Crackers, except potato-based ones	500	400
Crunchy bread called Knäckebrot	450	350
Gingerbread	1000	800
Products similar to other products in this category	500	300

These results are in agreement with those reported by Kermat et al. (2011) on bread products (especially on the yeast leavened ones) (4). Moisture content is a more important factor than temperature in order to control AA levels, a higher one leading to a lower level of AA, which must be also taken into account for the high variation between different types of cereal products (25, 26). However, due to its large consumption it is considered one of the most important sources of AA to total dietary intake.

As it may be seen in **Figure 2**, a large variety of the AA levels were recorded among the samples belonging to the same type of cereal products.

These high differences between the AA levels of the samples are due to their heterogeneity differences. For example, the category of biscuits included soft, dry, and hard as well as biscuits with different types of cream. The confectionery products type included different cake bases, cakes with nuts, and cakes without fruits, etc. All the samples from the biscuits and confectionery type were made from wheat flour in which various ingredients (including honey) have been added. It is known that within some categories of biscuits, those containing ammonium raising agents, inverted sucrose, sugar (sucrose) may present higher levels of AA (6, 9) and therefore, this big difference between samples among the same category type was an expectable one. The bakery products which presented the lowest level of AA included bread (yeast leavened ones) obtained from different ingredients such as wheat flour, rye flour, different types of fibers and seeds but rolls, pita and crisp breads were also analyzed. The processing conditions (time-baking temperature, fermentation time), the ingredients used (the amount of asparagine from the flours used, the reducing sugars levels, calcium supplementation) influence the AA level from the bakery samples (4).

The values obtained in Romania for the AA level in cereal products in 2017 period complied with the reference values for AA in cereal products established for this period by the European Commission (2013/647/EU). In 2017, the EC adopted new reference values for the AA content in food products throughout the European Commission 2017/2158/EU which came into effect on the 11th of April 2018. Therefore, in 2018, the data obtained for AA levels in cereal products on the Romanian market complied with this new EC (2017/2158/EU) established values. The levels for AA in different cereal products set by European Commission in 2013 and 2017 are shown in **Table 4**. These values are only indicative ones able to be achieved by the EU Member States and are not an indication of food safety or lack of it (18).

As you can see the established values in 2017 are significantly lower compared to the AA values recommended in 2013.

If the AA levels in foodstuffs exceed the AA values recommended by the EC, some investigations are required from the food producers on the processing methods and food production (27). Thus, measures can be taken in order to reduce AA levels in foodstuffs and therefore to regulate and control them. In the case of cereal products analyzed in Romania in 2017, only one biscuits sample exceeded the AA benchmark indicative value established by EC (2013/647/EU). From the 48 cereal products samples analyzed in 2018, 4 biscuits samples from the supermarket exceeded the reference levels established by EC (2017/2158/EU). The data obtained for the AA content from the cereal products on the Romanian market showed that only 9% from the analyzed samples were higher than the benchmark levels established by the European Commission. In our opinion, these data are relevant because, in Poland in a 2019 study from 40 cereal products analyzed, 30% of the tested bread, 80% of the tested breakfast cereals and 100% of the tested biscuits exceeded the benchmark levels of 2017/2158/EU. Also, the mean AA content in soft wheat bread, biscuits and breakfast cereals were 10%, 49% and 34% higher than the EC guidance levels (27). A study made in Italy also reported that 22.7% of biscuits and 33% of breakfast cereals were higher than the EC references levels (28).

Conclusions

This current study shows important informations on acrylamide level in cereal products from the Romania market with significance impact on public health and consumers. The number of cereal products samples analyzed to the national level in

order to establish its acrylamide content during the 2017-2018 period was only 55. This number of samples analyzed may be considered a very small one compared to the potential genotoxic and carcinogenic effects of acrylamide on the human body. The acrylamide data obtained in our study were reported to the European Regulations, for the acrylamide level from 2017 EC (2013/647/EU) and 2018 EC (2017/2158/EU). The results show that in general the acrylamide level from the cereal products are according to the European Regulations. It seems that a widespread potential risk from acrylamide for consumers occurred only in biscuits from which only 5 samples exceeded the reference levels established by the EU Regulations.

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Conflict of interest statement

The authors declare no conflict of interest.

Ethical compliance

This article does not contain any studies involving human participants or animals performed by any of the authors.

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