A kép illusztráció / Picture is for illustration only
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The acrylamide content of our foods

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1. Summary

It has been known since 2002 [5] that during the heat treatment production of foods that contain both carbohydrates and amino acids, acrylamide is also formed among the transformation products in a Maillard-type reaction, depending on the chemical composition of the raw materials and the temperature used in the technology. According to the literature, acrylamide may initiate carcinogenic processes in the human body.

In the paper, the Maillard reaction and the process of acrylamide formation is outlined. The biochemical significance of acrylamide is also discussed, as well as its toxic and carcinogenic effects on the human body. In 2017, manufacturers' measures aimed at decreasing acrylamide levels in heat treated, mainly baked, foods, as well as mandatory laboratory testing were regulated by a European Union Commission decree, and maximum permissible acrylamide levels in the foods in question were also set. The regulation to be applied from 11. April 2018. [9] In this connection, some laboratory test methods available in the literature will be described, including one that is based on a non-chromatographic principle.

Prior to the publication of the EU Commission decree, between 2006 and November 2017, the acrylamide contents of 250 drinking water samples, 715 potato chip samples and 67 other food samples (for a total of 1033 samples) were tested at the request of our partners. The limit of quantification (LOQ) of our analytical tests was 1.0 µg/L for drinking waters and 10 µg/kg for solid foods. Primarily the aim of this manuscript is to refer about investigation of solid food products, therefore the details of gas chromatographic analysis of drinking water samples will be only sketched as a brief completing information. It should be noted that, during the period in question, there were no legal limit values in the EU for acrylamide for solid food products.

2. Introduction

There is a compounds among the products of the chemical reaction discovered in 1912 by the French physician and biochemist Louis-Camille Maillard **[1]**, which has a detrimental effect on human health, according to decades of research. The acrylamide that forms in the Maillard reaction during the heat treatment of food raw materials that contain both amino acids and reducing sugars was most likely unknown to Maillard himself. The discovery and first synthesis of the molecule took place in 1949. Industrial and laboratory use of polyacrylamide, the polymer of acrylamide started in the 1950s **[2]**. Polyacrylamide as a water-soluble polymer is used in the purification of different waters, in sewage sludge treatment, in papermaking, soil stabilization and for other purposes as well. Polyacrylamide gel is a material indispensable for the gel electrophoresis technique used in the laboratories (PAGE) **[3]**. Therefore, the detrimental effect of acrylamide on human health, regarding occupational health, had been already recognized before the discovery of its appearance in foods **[4]**. The presence of the compound in bakers'

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wares was demonstrated in 2002 by a researcher Eden Tareke and his team during a research project carried out in cooperation between the University of Stockholm and the Swedish National Food Authority, when human exposure to different toxic compounds, including acrylamide from the environment, was tested by analyzing hemoglobin adducts in the blood **[5]**. In their research, it was found that in Sweden the average detectable hemoglobin adduct in the blood serum of the adult population corresponds to a daily intake of about 100 µg of acrylamide. Since the continued intake of this amount can lead to a risk of cancer, it was decided by the research team to explore the sources of this exposure of the population. It was found in their investigation that the acrylamide in the human body comes mainly from the consumption of bakers' food products **[5]**.

In the risk assessment report of the European Commission Joint Research Centre (EC JRC) published in 2002, the food safety risk of acrylamide that manifests itself through the food chain is not yet mentioned. However, the 221-page scientific work discusses in detail the adverse effect of acrylamide migrating from environmental elements, cosmetics, horticultural preparations, paper products, packaging materials and textile products **[6]**. According to the JRC report, acrylamide does not accumulate in the environment or the food chain, the toxic effect of the compound can only manifest itself directly, for example, after the consumption of contaminated drinking water **[7]**.

After the publication of the work of Tareke et al., **[5]** a large number of papers related to acrylamide appeared in the literature. When typing the word "acrylamide" in the Google search engine on February 24, 2018, there were 3,630,000 hits reported.

Since acrylamide was classified by the International Agency For Research on Cancer (IARC) as a group 2A compound (probably carcinogenic to humans), the permissible maximum levels of acrylamide in the workplace air of plants producing or processing this compound were limited to no more than 0.03 mg/m^3 **[8]**.

For the legal regulation of maximum permissible acrylamide levels in the different foods, one had to wait for six years after the publication of the IARC report. The regulation of the Commission of the European Union establishing mitigation measures and benchmark levels for the reduction of the presence of acrylamide in foods came into force on November 20, 2017 **[9]**.

With the entry into force of the regulation, it has become a legal obligation to implement comprehensive measures throughout the European Union to reduce the levels of acrylamide produced among the transformation products formed in the technology of foods produced by heat treatment from raw materials that contain both carbohydrates (reducing sugars) and proteins. The regulation contains detailed prescriptions for the modification of the technological steps in the production of foodstuffs involved in the formation of acrylamide, with special emphasis on the baking temperatures used, for the sampling of the products manufactured, and for the analytical performance characteristics of the laboratory tests of the food samples. Annex IV of the regulation contains the reference values (maximum permissible concentrations of acrylamide) for each food category concerned **[9]**.

In our laboratory, we have been carrying out acrylamide analyses in food samples since 2006, based on the available literature data and the because of the expected legal requirements. During the period prior to the publication of the relevant regulation, at the request of our partners, 1,033 samples were analyzed. The majority (715) of the samples were potato-based chips. In addition, the analysis of 250 water samples and 67 food samples of other type was requested by our partners. During the measurement process, gas chromatographic separation was used in the case of waters and high performance liquid chromatographic separation in the case of solid food samples. The acrylamide content of the prepared extracts was detected without derivatization, using a mass selective detector. For identification and the recording of the calibration curves, ^{13}C or D_3 isotopically labeled acrylamide certified reference material was used. The limit of quantification (LOQ) was 1.0 µg/L in the case of waters and 10 µg/kg in the case of solid foods. Our measurement results were below the LOQ value for all water samples. In the case of potato chips, the most common values were between the LOQ 1500 µg/kg.

3. Literature overview

3.1. The formation of acrylamide during the heat treatment production of foodstuffs

The appealing yellowish red or reddish brown color and the pleasant aroma of foodstuffs produced by heat treatment, especially baking, are the result of the reaction, described by Maillard, between the components of food raw materials containing amine and carbonyl groups, among other things **[1]**.

Unfortunately, the Maillard reactions that take place during the heat treatment of foods result not only in compounds with favorable organoleptic properties (pleasant fragrance and aroma components, pigments that provide a pleasing brownish color), but also compounds that are harmful to biological systems, including mutagenic and carcinogenic substances. The latter include acrylamide.

Acrylamide is a low molecular weight, highly water-soluble organic compound that is produced from naturally occurring ingredients, asparagine and

sugars, in certain foods when they are produced typically at temperatures exceeding 120 °C and at a low moisture content. It is produced primarily in carbohydrate-rich foods fried or otherwise baked, for example, in cereals, potatoes, coffee beans, the raw materials of which contain acrylamide precursors. In view of the carcinogenic properties of acrylamide, in the regulation the Committee prescribed that the acrylamide content of those foods has to be reduced, the raw materials of which contain the precursors of acrylamide (especially asparagine and reducing sugars) **[9]**.

Since the 1950s, there has been a large number of publications by food technology research institutes regarding the chemistry of the brown compounds that appear on the surface of foods produced by heat treatment (baking). According to the detailed study of Hodge, browning processes that take place during the heat treatment of systems containing sugars and amines can be divided into three main groups, and can be characterized by seven different chemical reaction types (from a to g):

1. Initial state (colorless compounds with no light absorption in the near UV range as well);
   a. Sugar-amine condensation;
   b. Amadori rearrangement;
2. Intermediate phase (colorless or yellow color, strong light absorption in the near UV range);
   c. Dehydration of sugars;
   d. Fragmentation of sugars;
   e. Amino acid decomposition;
3. Final phase (appearance of strong colors);
   f. Aldol condensation;
   g. Aldehyde-amine polymerization, formation of heterocyclic nitrogen compounds.

According to the publication of Hodge, already in the 1950s, the formation of more than 100 compounds in the chemical reactions during the heat treatment of foods was confirmed. His paper will not be described in detail, but it should be noted that it is an indispensable source for the in-depth study of the Maillard reaction **[10]**.

The complexity of the complicated processes that take place during baking is confirmed by a literature data: up to the 1990s, about 250 bread aroma components had been isolated and identified by gas chromatographic techniques, but this still was not enough to decipher the "secret" of bread aroma **[11]**.

So, in foods produced by heat treatment, reducing sugars react with compounds possessing free amino groups, and at sufficiently high temperatures (120-180 °C) various aroma components and brown-colored melanoid compounds are formed. In the literature, the formation of colorants in the Maillard reaction is called non-enzymatic browning. During this process, the free amino group of the amino acids present is additioned to the aldehyde or ketone carbonyl group of reducing sugars. Subsequently, the amino group is converted into an imine group by the elimination of water, and glycosylamine type compounds are formed. In the presence of proton, imonium ions are formed from the imine compounds and, depending on the starting sugar type, an intramolecular rearrangement occurs. In the case of an aldohexose starting material (e.g., glucose) an Amadori rearrangement, in the case of a ketohexose (e.g., fructose) a Heyns rearrangement takes place. The two processes are shown schematically in ***Figure 1*** **[12]**.

The steps of the Maillard reaction between asparagine and a sugar molecule containing a carboxyl group are shown schematically in ***Figures 2/a*** and ***2/b***, with the formation of a Schiff base.

As shown in ***Figure 2/b***, the Maillard reaction has at least two pathways along which acrylamide may form. One is the process between the temporarily appearing Amadori product and a reducing sugar, resulting in acrylamide via a 3-aminopropionamide intermediate. The other pathway is the reaction between another amino acid molecule and the Amadori product **[13]**. Amadori products can be detected in a variety of preserved foods as well (e.g., dried fruits, vegetables, milk powder). Since the amino acids bound in the Amadori products are inaccessible to the digestive enzymes in the alimentary tract of warm blooded animals, they cannot be utilized as nutrients, and so the formation of such compounds reduces the protein value of foods. The majority of the hundreds of products formed in the Maillard reactions are pigments, and a smaller part are aroma compounds. It should be noted that hydroxymethylfurfural (HMF), forming in foods – namely honeys – in limited quantities, is also a product of this reaction family **[12]**.

According to Grob, the intensity of acrylamide formation in heat-treated foods is mostly related to the asparagine, aspartic acid and fructose contents of the raw materials. Compared to fructose, less acrylamide is produced in the amine addition of glucose. Lactose has an effect that is 10 to 15 times smaller than that of fructose. In the case of potato-based foods, the food safety risk is primarily due to the high asparagine and reducing sugar contents. Depending on the variety, potatoes contain 2.0 to 4.5 g asparagine per kg of original matter, corresponding to 10-20 g/kg dry matter. Wheat flour contains about 50 times less asparagine compared to potatoes. Therefore, in the crust of breads baked to a dark color, the amount of acrylamide will be <200 mg/kg, while in the skin of potatoes baked to a dark color the amount could be 2,000-10,000 mg/kg. Ammonium ions in the raw materials (e.g., in baking powder) increases the amount of acrylamide produced. ***Table 1*** shows the data collected by the Swiss

Federal Office of Public Health, obtained by testing potato products **[14]**.

The amount of acrylamide produced in each baked product depends also on the plant variety used, the baking temperature and the moisture content of the end product. These relationships are illustrated in ***Figure 3*** **[15]**.

Figure 3 shows that the formation of large amounts of acrylamide can be expected when using potatoes as raw material, a baking temperature above 150 °C and in the case of low moisture content of the end product. The professional material from 2008 and the provisions of the Community decree that came into force in 2017 are in good agreement. The Commision regulation of the European Union mentions specifically the monitoring obligation of products manufactured using baking technologies over 160-170 °C. In addition, a moisture content of at least 1% is prescribed in the case of potato chips **[9]**.

The temperature dependence of the formation and decomposition of acrylamide has been analyzed in several papers. Of these, I would like to quote two remarkably similar diagrams from two authors. ***Figure 4*** shows the formation/decomposition curve as a result of baking potatoes, given in Weiβhaar's presentation **[19]**.

The temperature dependence of acrylamide formation and decomposition in the case of baking potato products is illustrated by Grob in another lecture material based on his own measurement results. ***Figure 5*** shows that, without the addition of sugars or ammonium ion containing additives, the resulting transformation product starts to decompose above 180 °C. If the starting materials include an ammonium-containing additive, then acrylamide formation starts already at 60 °C, reaches its maximum between 100 and 140 °C, but with increasing temperature the product of the Maillard reaction starts to decompose rapidly, however, its amount is still expected to be around 2000 µg/kg even above 180 °C **[16]**, which is several times higher than the current legal limit value of 750 µg/kg **[9]**. The two figures have been placed side to side in order to illustrate that serious acrylamide formation can be expected when baking potato raw materials, and to show that similar formation dynamics had been observed independently by two researchers in Germany and Switzerland. The difference between the two curves is that while Weiβhaar carried out the experiments using only potatoes, sugars and ammonium-containing baking agents had been added to the experimental products by Grob. Grob's experiments show that, in the presence of ammonium ion, acrylamide amounts exceeding 10,000 µg/kg may form already at 120 °C, to reach the same concentration, a temperature of at least 160 °C is necessary in the absence of additives.

It was demonstrated by Muttucumaru et al., by examining products made from 12 different varieties of potatoes, cultivated in the United Kingdom around Doncaster and Woburn, that the chemical composition of the same potato varieties may differ significantly, especially in terms of the asparagine and sugar ingredients, depending on cultivation conditions. Consequently, the acrylamide content of the foods made from them may also differ significantly. According to their study, when baking for 20 minutes at 160 °C, the ratio of reducing sugars in the potatoes (mmol/kg) and the acrylamide formed during baking (µg/kg) is approximately 1:2.3. The correlation between these two variables is significant ($r = 0.516$, $p < 0.001$). The asparagine content (mmol/kg) and the amount of acrylamide formed (µg/kg) can be characterized by the same 1:2.3 ratio, with a correlation greater than the one mentioned above ($r = 0.672$) **[18]**. It should be noted that the relative concentration ratio of the three components (asparagine, reducing sugars, acrylamide) is stochastic, yet it characterizes clearly the food safety situation arising when baking potatoes. To those interested in the topic I highly recommend a detailed study of literature source **[18]**, concerning the relationship between asparagine, reducing sugars, cultivation sites, varieties and the amount of acrylamide produced. The paper also presents valuable diagrams of the results of potato storage experiments, which cannot be included here because of the limited scope of this manuscript.

3.2. The acrylamide content measured in some food groups

It is difficult to assemble a consistent picture from the literature data reporting the acrylamide content of different food groups. Food groups to be included in the surveys are (were) designed by the food chain safety organizations and research communities of the various countries by taking into consideration national and regional characteristics and local consumer habits. Therefore, to give you a taste of this, data from an American, a Chinese and a Hungarian source are presented, in addition to the analytical results of the Rapid Alert System for Food and Feed of the European Union (RASFF) **[22]**.

In 2004, it was found by Viator and Muth, based on the results of the FDA survey carried out in the USA, that the largest acrylamide exposure was caused by the consumption of potato-based foods. ***Figure 6*** shows the measured highest and lowest values of the acrylamide contents of the food groups investigated by the FDA. The numerical values of ***Figure 6*** are summarized in ***Table 2*** **[20]**.

Between 2010 and 2015, the EU Panel on Contaminants in the Food Chain has processed 43,499 analytical results of foods coming from 24 European countries. According to estimation carried out using statistical means, the highest acrylamide

levels were measured in coffee substitutes and coffee products, with concentrations of 1,499 and 522 µg/kg, respectively. The average acrylamide level of potato-based chips and similar products was 389 µg/kg. In fried potatoes, the value was 308 µg/kg. The lowest values were found in cereal-based products intended for infants, with an average of 73 µg/kg. Within the framework of the survey, based on the change over time in the analytical results of 40,455 chips made from freshly sliced potatoes from 20 countries, it was determined that the acrylamide content of these products had decreased significantly between 2002 and 2011: from 763 ± 91.1 µg/kg to a value of 358 ± 2.5 µg/kg. Decreasing concentration over time were also found in the case of other product groups. It was found that in Europe, the estimated acrylamide exposure of 95% of the pediatric population was in the 0.5-3.4 µg/kg body weight range. Values differed significantly within this range in different survey campaigns. For 95% of the adult population, the estimated value was in the 0.4-2.0 µg/kg body weight range. Results for adults have also changed from survey to survey, and depended significantly on the age of the population under study. It was found that the amount of the acrylamide produced in meals and foodstuffs, for example, when using potatoes as a raw material, is largely dependent on the variety and on cultivation site conditions **[17]**.

Measurable amounts of acrylamide was found in 115 of 123 food groups selected by Chinese researchers. In most of the food groups investigated, the acrylamide content was below the 500 µg/kg value, but the highest concentration in fried potatoes, rounded to the nearest whole number, was 4,126 µg/kg. For this food group, the average value was calculated to be 604 µg/kg, with a significant standard deviation (SD = 1,328 µg/kg). Results of their study, rounded to the nearest whole number, are shown in ***Table 3***. Minimum and maximum values of the table are shown in ***Figure 7***.

Table 4 contains acrylamide levels published in the University of Szeged lecture notes after Kiss and Toldi **[28]**. Values of the table are shown in ***Figure 8***. When comparing the date from the three sources **[26]**, **[27]**, **[28]**, it is quite conspicuous that the distribution of the minimum and maximum values published in the American and Hungarian papers, without statistical confirmation, can be considered similar. From the Chinese data, it is likely that the consumption of foods roasted to a dark color is less common in Chinese cuisine, but foods made from potatoes are fried at high temperatures.

On March 2, 2018, only 20 notifications related to acrylamide content could be found on the RASFF Portal. The small number of RASFF entries can be explained by the fact that it was not mandatory to apply the provisions of the decree that came into force on November 20, 2017 **[9]** at the time when this paper was completed, therefore, no authority proceedings could be possible for the presence of acrylamide exceeding the limit value before April 11, 2018. Simplified data for RASFF notifications are summarized in ***Table 5*** **[23]**.

Looking at the date in ***Table 4*** we can see that in eight years, an astonishingly small number of entries, alerts and events requiring follow-up were recorded in the RASFF system. Most of the notifications were submitted by Croatia due to the acrylamide content of biscuits from Bosnia and Herzegovina (BIH) and Serbia (SRB). In addition to Croatia, one case each was reported by the Netherlands, Belgium, Malta and Poland. Compared to the other cases, the notifications by the Netherlands and Belgium were because of significantly higher acrylamide contents, with values of 2,308, 4,757 and 2,062 µg/kg. Outstandingly high values of 5,000 and 5,900 µg/kg of potato chips flavored with sea salt from the United Kingdom indicate that, in 2009, the health-damaging effect of acrylamide was still not taken seriously by certain food manufacturers and probably did not feel sufficiently large responsibility for the population consuming their products. Similarly high values, also in potato chips, were found among the Chinese data **[21]**.

If the acrylamide-temperature data in ***Figures 4*** and ***5*** are analyzed **[19]**, **[16]**, it can be understood that Maillard reactions that take place during the baking of potato products can result in dangerously high amounts of transformation products in case of manufacturing technology non-conformities.

Based on the data in ***Section 3.2*** it can be assumed clearly that today the acrylamide intake of the population comes mostly from the consumption of foods made from potatoes by frying (chips, fried potatoes), in case of American, Asian and European foods as well.

According to Zhou, in addition to environmental effects and foods, cigarette smoke can also be a source of acrylamide for smokers **[31]**.

3.3. Human health effects of acrylamide

The adverse effects of acrylamide on human health had already been known to occupational health experts before 2002. Toxicologists had already performed acute toxicological experiments on warm-blooded animals in the 1980s. Prior to the discovery in Sweden **[5]**, the molecule had been known to enter the human body as a powder or vapor **[28]**. In the non-food sector, workers with chronic contact with acrylamide experienced sensory disturbances in the hands and feet **[23]**. Prolonged contact of the compound with the skin may cause a reddish-blue discoloration **[24]**. As its effect, reflex responses of the individual may weaken **[25]**, peeling of the skin may start, the hands and feet may exhibit a reddish discoloration, and teratogenic effects were

also observed in warm-blooded animals **[26]**. The neurotoxicity of the compound is indicated by the fact that, in the case of acute exposure, drowsiness, numbness, muscle tremors, and in the case of severe poisoning, speech disturbances may occur **[27]**.

Its per oral LD_{50} value in rats is 159-300 mg/kg body weight. In the case of rabbits, the skin LD_{50} value is 1,680 µL/kg body weight. When administering subacute doses of 20 mg/kg body weight/day, peripheral neuropathy, atrophy of the skeletal muscles and a decrease in red blood cell counts were observed in rabbits. Monkeys exhibited similar symptoms when treated with 10 mg/kg body weight/day doses for 12 weeks. The genotoxicity of the compound is demonstrated by the fact that when mice were fed feedstuff containing 500 µg/kg acrylamide for 3 weeks, breakage of sister chromatids and the exchange of certain gene sequences became common. When adding 3 mg/kg body weight/day acrylamide to the drinking water, the prevalence of tumorous lesions increased in the adrenal glands, the thyroid, the nasal cavity and, in female animals, in the mammary and the uterus, and in male animals in the testicles **[28]**.

Acrylamide can react in biochemical systems in two ways: one is the reaction of the electrophilic C=C conjugated double bond, the other is the reaction of the amide group. When reacting with hydrogen-containing compounds, the C=C bond is converted to glycidamide by cytochrome P450 enzyme reactions in the liver. Glycidamide and acrylamide itself have electrophilic properties and therefore react with the sulfhydryl groups of proteins and hemoglobin, forming adducts **[28]**. The resulting chemical structures were detected by Tareke and his team in 2002 in the blood serum of individuals exposed to acrylamide **[5]**. Since adduct formation occurs also with nucleosides (adenosine, guanosine, cytidine, etc.), even though with low efficacy **[28]**, the mechanism of genotoxicity of the compound becomes apparent as well.

The genotoxic effect of acrylamide was confirmed by Neuhäusser-Klaus and Schmahl using spot tests and inducing specific locus mutations. In the experiments, lesions in spermatogonial stem cell cultures (cells that produce male reproductive cells) were evaluated by comparing acrylamide to 14 other compounds. As a result of their experiments, in the case of both methods, large deletions, loss of chromosomes and somatic recombinations were observed in the hereditary material of the cells. The mutagenic and teratogenic effects of acrylamide were confirmed by morphological and histological studies carried out on the 17-day-old fetuses of experimental animals treated with the compound. According to acute studies, a 30 mg/kg body weight acrylamide dose in mice doubles the frequency of natural genetic disorders in fetuses **[29]**. According to Russel et al., acrylamide-induced genetic mutations in the hereditary material of male warm-blooded animals may be permanent, and so the mutagenic effect caused by the compound may be inherited by the offspring of exposed organisms. Similar genetic disorders in female animals could not be confirmed by their research results **[30]**.

Je el al. in their cohort study investigated data from 453,355 female person. Among them 2,019 women suffered in endometrial cancer. The illness wasn't associated with dietary intake of acrylamide in the whole population – including the smoking women – but among the never smoked patients the risk of cancer significantly increased with high dietary acrylamide intake **[32]**. As my opinion the harmful components of cigarette smoke can mask the physiological influence of acrylamide intake in the case of smoking patients. It may be the explanation of the significant correlation between cancer risk and the high acrylamide intake.

Another characteristic of the genotoxic effect of acrylamide is the disruption of cell division and the replication of the hereditary material. The compound also attacks the SH group of topoisomerase II enzymes **[33]**. Topisomerase enzymes are involved in the regulation of DNA cleavage. They relieve the torsional stress generated in the DNA strand unpackaged by the helicase enzyme. This ensures the spatial structure that is indispensable for the flawless operation of uncoiling and rewound DNA strands in dividing cells. Their inhibition causes irregular epigenetic changes (permanent modification of the genetic material) **[34]**. Toxic substances that are able to fix the temporary covalent bonds between the DNA and the topoisomerases are called topoisomerase inhibitors. By disrupting the replication of the hereditary material, topoisomerase inhibitors cause the death of dividing cells or their abnormal growth **[35]**. Sciandrello et al. investigated the effect of acrylamide in a V79 cell culture from Chinese hamster. According to the results of their experiments, acrylamide may reduce the effect of certain topoisomerase poisons, such as the Etoposide used in chemotherapy, but it also inhibits the functioning of the enzymes itself, and so it can be considered a topoisomerase inhibitor. If dithiothreitol (a vicinal diol containing two SH groups: $C_4H_{10}O_2S_2$) was added to the cell culture treated with acrylamide, the topoisomerase inhibitory effect of acrylamide was reduced. Based on this, it is assumed that acrylamide targets not only topoisomerase enzymes, but also other enzymes containing SH groups that are involved in cell metabolism **[33]**.

The available scientific literature provides abundant material to understand the mechanism of action of the genotoxicity of acrylamide. The few experimental results described in this section indicate that this compound is capable of attacking processes at the molecular biological level in multiple locations. Based on this, it was justified for the Commission of the European Union to take steps in order to reduce the amount of acrylamide in foodstuffs intended for public consumption.

3.4. Acrylamide regulation in the European Union – history and regulation

The Scientific Panel on Contaminants in the Food Chain of the European Food Safety Authority (EFSA) adopted a declaration on the acrylamide content of foods on 19, April 2005, endorsing the risk assessment of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) of February 2005 on the acrylamide content of foods. In this assessment, JECFA found that, in the case of average and high-volume consumers, exposure limit values were low, considering that acrylamide is a genotoxic and carcinogenic compound, which is a cause for concern from a human health point of view. Therefore, continued efforts are needed in order to reduce the amount of acrylamide in foods. Based on the 2005 declaration of EFSA, in this spirit, the first recommendation of the Commission of the European Union in order to monitor acrylamide levels was published on 3, May 2007 **[36]**.

Between 2007 and 2010, the Confederation of Food and Drink Industries (CIAA) has developed an action plan for food manufacturers with tools that could effectively reduce acrylamide levels in their products. However, in 2009 it was found that food manufacturers' measures aimed at reducing acrylamide levels were insufficient. Therefore, the recommendation of the Commission was revised in 2010, and the food chain safety organizations of the member states were ordered to perform further monitoring studies. The testing of a total of 2,042 products in 10 product groups was requested from the member states **[37]**.

The breakthrough came with the announcement of the Commission regulation that came into force on November 20, 2017 **[9]**. It was declared that acrylamide is a contaminant according to the definition of Article 2 of Council Regulation (EEC) No 315/93, and as such, constitutes a chemical hazard in the food chain. Foods that contain a contaminant in unacceptable quantities from a health and, in particular, toxicological point of view, may not be placed on the market. In addition, the level of contaminants must be kept as low as can be reasonably reached by observing good practice **[38]**.

The regulation contains detailed specifications for the technological characteristics of foods that are expected to have a high acrylamide content (agrotechnology, storage conditions, preparation of raw materials, baking temperatures, recommended minimum moisture content, etc.), and also maximum permissible acrylamide levels. The main food groups listed in the Regulation are the following **[9]**:

- French fries, other cut (deep fried) products and sliced potato crisps from fresh potatoes;
- potato crisps, snacks, crackers and other potato products from potato dough;
- bread;
- breakfast cereals (excluding porridge);
- fine bakery wares: cookies, biscuits, rusks, cereal bars, scones, cornets, wafers, crumpets and gingerbread, as well as crackers, crisp breads and bread substitutes. In this category a cracker is a dry biscuit (a baked product based on cereal flour;
- coffee (roast coffee and instant coffee);
- coffee substitutes;
- baby food and processed cereal-based food intended for infants and young children as defined in Regulation (EU) No 609/2013 of the European Parliament and of the Council.

Table 6 summarizes the benchmark levels of Annex IV of Regulation 2017/2158 **[9]**. Details of the regulation regarding the benchmark levels of breakfast cereals, coffee substitutes and infant formulas are not included in ***Table 6***.

Prescriptions of Commission Regulation (EU) 2017/2158 establishing mitigation measures and benchmark levels for the reduction of the presence of acrylamide shall apply from 11, April 2018.

3.5. Analytical methods

Collected literature references regarding the analysis of acrylamide are only outlined briefly. Detailed methods can be studied in the referenced source works.

To determine the acrylamide discovered in foods, gas chromatographic and liquid chromatographic techniques were used by Tareke et al. **[5]**.

For gas chromatography measurements, acrylamide was extracted from 10 g of sample with 100 mL of water using a laboratory blender. To the filtered extract N,N-dimethylacrylamide internal standard was added. Prior to the instrumental analysis, the compounds were derivatized with bromine overnight at 4 °C, and the excess bromine was decomposed using sodium thiosulfate. After the disappearance of the yellow color of bromine, sodium sulfate was added to the reaction mixture and the brominated derivatives were extracted using a 1:4 mixture of ethyl acetate and hexane. The resulting solution was concentrated by evaporation and the final volume was adjusted to 200 µL. Sample components were separated using a temperature program and a 30 m × 0.25 mm capillary column with a film thickness of 0.25 µm (BPX-10 fused silica), and then they were detected by a mass selective detector.

In the liquid chromatography analysis, 1 mL of a 1 µg/mL concentration $^{13}C_3$ isotopically labeled acrylamide internal standard was added to the aqueous extract prepared in the above-described way. The extract was centrifuged twice, and then 3 mL of it was applied to an SPE column (Isolute Multi-

Mode SPE column, 300 mg) activated with acetonitrile and washed twice with water. The first 1 mL of the eluted samples was discarded and the rest was filtered through a 0.45 µm pore size filter. Following this, 500 µL of the solution was passed through an ultrafilter (Millipore Microcon YM-3). Liquid chromatographic separation was carried out on 200 µL of the sample on a (50 × 2.1 mm, 5 µm) ThermoHypersil column. For detection, an MS/MS system was used with a positive ESI ion source. Acrylamide was identified in an MRM (Multiple Reaction Monitoring) system. The $[M+H]^+$ precursor ion with a mass number of 72 fragmented with 54 $[H_2C=CH-C=NH]^+$ (collision energy: 16 eV) and 55 $[H_2C=CH-C=O]^+$ (collision energy: 11 eV) m/z transitions. The intensity of the ions was 1:35, with a ±20% uncertainty. For quantitative determination, data of the 55 m/z (mass/charge) transition were used and the calibration curve was recorded in the 10 – 5,000 µg range. The limit of detection (LOD) was 5 µg/kg in the case of gas chromatography measurements and 10 µg/kg in the case of liquid chromatography analyses, with recoveries close to 100% and a standard deviation of about 5% **[5]**.

A tandem LC/MS/MS system was used by Liu et al. for the measurement of the acrylamide content of tea samples. As an internal standard, they also used a $^{13}C_3$ isotopically labeled molecule. Extraction was carried out at room temperature with 10 mL of water in the first step and 10 mL of acetonitrile in the second step. 9 mL of the acetonitrile extract was concentrated to 0.5 mL, and the acrylamide was bound on an Oasis MCX SPE column. The LOD value of the instrumental analysis was 1 ng/mL, the limit of quantification (LOQ) was 5 ng/mL, and the recovery was between 74 and 79%. With the method developed for the analysis of teas, a detection limit (LOD) of 20 ng/g (20 µg/kg) was achieved with respect to the original matter. Thus, their results were between the LOD and 100 µg/kg **[39]**.

Ishizuka and his team analyzed polyacrylamide in samples of cosmetic products, among other things. According to their report, their method may be suitable for the analysis of food samples as well. For the preparation of polyacrylamide samples, a combines solid phase microextraction and head space analysis (HS/SPME – Head Space/Solid Phase Micro Extraction) was developed. After mixing with a 2-methylacrylamide internal standard, aqueous polyacrylamide samples were thermostated with stirring at 60 °C for 10 minutes, and then the compounds in the head space were adsorbed for 60 minutes onto a CAR/PDMS (Carboxen-polydimethylsiloxane) fiber. Acrylamide polymers bound to the fiber were desorbed in the splitless injector of a gas chromatograph equipped with a nitrogen-phosphorus detector for 10 minutes at 200 °C. Separation was carried out in a temperature-programmed mode using a 30 m × 0.25 mm 0.25 µm ZB-WAX fused silica column. The calibration curve was recorded in the 0.1-2.0 µg/mL range from a solution containing 20% polyacrylamide. To determine the recovery, 100 µL (10 µg/mL concentration) aqueous acrylamide solution was added to 1 mL of 25% polyacrylamide solution, together with 2-methylacrylamide internal standard (50 µg/mL). In addition to the nitrogen-phosphorus detector, alternatively, a mass selective detector was also used for the determination of acrylamide, using a 60 m × 0.25 mm, DB-Wax column with a film thickness of 0.25 µm **[40]**.

The determination of acrylamide in foodstuffs is a rather uncomfortable task because of its low molecular weight (71.08 Da), lack of chromophoric properties, high polarity, good water solubility, pronounced reactivity and low volatility. For the gas chromatographic separation used in the laboratories, brominated derivatives have to be prepared. There are numerous compounds that are extracted together with acrylamide which interfere with liquid chromatographic separation. Application of the Quadrupol-Orbitrap hybrid mass analyzer coupled with a high resolution HPLC (HPLC-Q-Orbitrap) may provide further analytical possibilities. Extracts obtained by a clean-up based on the QuEChERS (Quick Easy Cheap Effective Rugged Safe) method were separated by Pugayeva et al. **[41]** on a 100 × 3.0 mm Phenomenex column with a 5 µm stationary phase (Torrance, USA) using gradient elution on a UPLC instrument. Eluent A was a 0.1% aqueous solution of formic acid, while eluent B was a 0.1% acetonitrile solution of also formic acid. 10 µL of the extracts was injected into the loop of the HPLC instrument. Using their method, the lowest validated acrylamide level in roasted coffee was 10 µg/kg.

The ion source of the system was a positive ionization mode, high-temperature electrospray unit (H-ESI-I – Heated Electrospray Ionisation). The m/z value of the precursor ion was 72.0444 Da, while in the case of the D_3-acrylamide used as an internal standard it was 75.0632 Da (remember, we are talking about a high resolution MS system). The scan range was set to between 50 and 80 m/z.

Homogenized samples were extracted using 10 mL of water and 10 mL of acetonitrile. Magnesium sulfate and sodium chloride was added to the extracts, they were centrifuged after vigorous shaking, and then 200 mg of PSA (Primary Secondary Amine) and 600 mg of magnesium sulfate was added to the acetonitrile extract. The mixture was centrifuged after vortexing, and then 2 mL of the supernatant was passed through a Strata NH_2SPE column which had been activated with 3 mL of acetonitrile and then vacuum-dried, without the use of a vacuum. HPLC-Q-Orbitrap separation was carried out on the eluate dripping from the SPE column. For the separation, a hydrophilic interaction column (HILIC – Hydrophilic Interaction Chromatography) was used. Mass spectrometric detection was performed in targeted MS2 mode. The ions to be analyzed were screened

by the Quadrupol unit, they were fragmented in the a HCD (High Energy Collision Dissociation) cell, and were collected in the C-trap (Cross-section trap) unit. For identification and quantitative determination, the 72.0 → 54.9 and 75 → 58.0 m/z transitions were used (the latter being the transition of the D_3-acrylamide internal standard). The calibration curve was recorded in the 10 – 500 µg/kg range. Spiked acrylamide contents of the different coffee samples could be measured reproducibly for three days in the 10, 50, 200 and 450 µg/kg range. Average RSD_r values were 7.8, 4.6, 3.5 and 2.3% for the samples spiked with increasing concentrations and with 6 repetitions. Recovery was in the 100-111% range. When analyzing real coffee samples, recoveries were between 99 and 109%. During their work, 22 coffee samples were analyzed. The acrylamide contents of differently roasted coffee samples were in the 166-503 µg/kg range **[41]**.

To determine the acrylamide content of foods with a high fat content is a difficult task because of the high polarity of the compound and its reactivity. Acrylamide is sensitive to high temperature, ultrasound, pH, the degradation activity of microorganisms and to co-extractable interfering compounds. Li et al. **[42]** chopped up various food samples (potato crisps, potato chips, cracker) using a meat grinder, they were homogenized and fat was removed by n-hexane. Fat-free samples were extracted with a 2 mol/L aqueous sodium chloride solution. The extracts were centrifuged, and the supernatant were clarified using 100 µL each of Carrez I and Carrez II solutions. After clarification, the extracts were again centrifuged, and then the supernatant was extracted with ethyl acetate. Water was added to the organic phase and the volume was reduced to 500 µL in a stream of nitrogen (note: acrylamide may decompose if the solution is evaporated to dryness). The final volume was adjusted to 2.0 mL with water. The contaminants of the extract were removed by passing through an Oasis HLP (6 ml, 200 mg) SPE column, and finally the extract was filtered through a 0.22 µm pore size filter disc. In the case of samples with higher fat contents, better recovery values were obtained by using an Oasis MCX (3 mL, 60 mg) SPE column for the clean-up.

Separation, identification and quantitative determination was carried out on a UHPLC-MS/MS system using a Chrome-Matrix C18 (2.6 µm, 2.1×100 mm) column. Separation was performed in gradient mode. The starting solution of the linear gradient elution was a 0.1% formic acid solution containing 1.0-15% methanol. At the end of the separation, the column was washed with a 0.1% formic acid solution in 15-100% methanol.

Separations were also carried out on an Atlantis dC18 (3 µm, 4.6×150 mm) column. In this case, the linear gradient was produced by adding 80-100% methanol to the 0.1% formic acid base solution. After the separation, the column was washed with 100% methanol. According to the authors, the analytical performance of the Chrome-Max C18 (2.6 µm, 2.1×100 mm) column proved to be superior in their experiments.

The separated molecules were ionized in a positive ESI source, and mass spectrometric data were collected in the MRM mode. The recorded calibration curve was linear in the 2 – 800 ng/mL range. When using a Chrome-Matrix C18 (2.6 µm, 2.1×100 mm) column, the LOD value of the measurements was 2 µg/kg and the LOQ value was 4 µg/kg. The relative standard deviation (RSD) of the measurement results did not exceed 3.3%. Average recovery was above 89% **[42]**.

For the determination of acrylamide in foods, not only chromatographic techniques are suitable. A method based on the fluorescence immunoassay principle was developed by Hu and his working group **[43]**, using an electrochemical biosensor. The method is based on the change in the fluorescent light emission of water-soluble amino-CdSe/ZnS (λ_{em}=600 nm) semiconducting nanoparticles (QD = Quantum Dot) modified with N-acryloxy-succinimide (NAS-QDs). The NAS-QDs complex was polymerized with UV light in the presence of a diphenyl-(2,4,6-trimethylbenzoyl)-phosphine (TPO) photoinitiator. The polymer itself has a low fluorescence emission. If acrylamide was added to the system, the fluorescence of the polymerized NAS-QDs complex increased significantly. During the measurement, acrylamide was extracted from the sample using the same method as in the case of the HPLC technique. The LOD value of the method was 3.5×10^{-5} g/L (35 µg/L), which satisfied the sensitivity requirements of the analyses of potato chips and various cookies. A particular advantage of the method is that, with the exception of L-asparagine, molecules with structures similar to that of acrylamide do not interfere with the measurement **[43]**.

4. Materials and methods

4.1.1. Literature sources of the methods used in the WESSLING laboratories

The method used for the detection and quantitative determination of acrylamide in foods in the laboratory of the Food Safety Business Unit of WESSLING Hungary Kft. was based on the publications of Hoenecke et al. **[44]**, and Mastovska et al. **[45]**.

Using one of the methods published by the Hoecke research group, various foods such as mashed potatoes, French fries, cereals, bread and roasted coffee samples can be conveniently tested. Their other method consisting of more complicated procedures can be used for the analysis of more complex matrices, such as cocoa, instant coffee, molasses or malt. Prepared samples were analyzed using a GC-MS/MS technique. In the mass spectrometric analysis, a sensitivity of 1 µg/kg could be achieved using the two

mass transitions of acrylamide. In routine laboratory practice, an LOQ of 5 µg/kg can be achieved using the gas chromatography technique and 30 µg/kg using the liquid chromatography technique. The variation coefficients of the measurements were in the 3–12% range, depending on the matrix **[44]**. In the method of Mastovska et al., extraction is performed using a mixture of acetonitrile and water, and then n-hexane is used for fat removal. During the extraction, acrylamide dissolves in the acetonitrile phase. After shaking the extract and short centrifugation, the supernatant n-hexane solution is discarded and PSA (Primary Scondary Amine) sorbent and anhydrous magnesium sulfate is added to 1 mL of the acetonitrile solution. The resulting extract is applied to the separation system. For mass spectrometric analysis, a positive ESI source is used **[45]**.

International standard EN 16618:2015 was adopted by the Hungarian Standards Institution in 2015 for Determination of Acrylamide in Food by Liquid Chromatography Tandem Mass Spectrometry (LC-ESI-MS/MS), and it was published in August 2015 **[46]**. Our accredited analyses are performed according to the prescriptions of this standard.

4.1.2. Instruments and equipment

- 50 mL and 10 mL polypropylene centrifuge tubes,
- Horizontal shaker,
- Vortex mixer,
- Centrifuge,
- Volumetric flasks with volumes of 5, 10 and 50 mL,
- Calibrated automatic pipette (100-1000 µL),
- Hamilton syringes (5,100, 500, 1000 µL),
- Analytical balance (0.1 mg precision),
- HPLC instrument with tandem mass selective detector.

4.1.3. Chemicals, reference materials

- Formic acid (HPLC grade),
- n-Hexane (HPLC grade),
- Acetonitrile (HPLC grade),
- HPLC grade water (e.g., Milli Q),
- Magnesium sulfate, anhydrous,
- Sodium chloride,
- PSA (Primary Secondary Amine sorbent),
- Eluent A: 1 mL formic acid + 5 mL acetonitrile in 1 L Milli Q water,
- Eluent B: acetonitrile,
- SPE columns (multimode polystyrene-divinylbenzene apolar strong anion and cation exchanger, 1000 mg/6 mL),
- Acrylamide standard (Sigma-Aldrich catalog no.: A3553),
- D_3-Acrylamide standard (Sigma-Aldrich catalog no.: 636568).

4.1.4. Sample preparation using our own analytical method

Analyses started with 2 g of sample. In a 50 mL volume lockable centrifuge tube, reference solution corresponding to 500 µg acrylamide, internal standard solution corresponding to 500 µg D_3-acrylamide and 5 mL of n-hexane were added to the spiked sample and it was shaken thoroughly. 10 mL of Milli Q water, 10 mL of acetonitrile, 4 g of anhydrous magnesium sulfate and 0.5 g of sodium chloride were added to the mixture and, after shaking for 5 minutes, it was centrifuged at 3700 rpm for 10 minutes.

After centrifugation, 1 mL of the acetonitrile phase was pipetted into a 10 mL centrifuge tube containing 50 mg of PSA and 150 mg of magnesium sulfate. After vortexing for 10 seconds, the content of the tube was centrifuged at 3700 rpm for 2 minutes and then the phase containing the acrylamide was pipetted into an amber vial, it was sealed and stored refrigerated until the measurement.

4.1.5. Sample preparation according to standard MSZ EN 16618:2015

Since the referenced source **[46]** is readily accessible to everyone, therefore, the extraction steps of the standard analysis are outlined in a diagram. ***Figure 9*** shows the steps of the standard extraction procedure of bakers' products and potato-based products. According to the standard, bakers' wares and potato-based products can be extracted without separate fat removal, but in the case of coffee samples the n-hexane step is necessary.

Further clean-up of the aqueous extract of ***Figure 9*** were carried out using 2 SPE columns. Clean-up steps on the solid phase extraction columns are described in ***Figures 10/a*** and ***10/b*** for bakers' wares, potato-based and coffee products (note: no real coffee samples were received).

SPE column no. 1 used for the clean-up of the extracts was conditioned with 3 mL of methanol and then with 2 × 6 mL of water before use. Conditioning of SPE column no. 2 was carried out using 5 mL of methanol and then 5 mL of water. The sample was allowed to pass through the column at a rate of 30 drops/minute without the use of vacuum. For chromatographic separation and quantitative determination, the volume of all extracts was adjusted to 500 µL using a block thermostat no warmer than 40 °C.

4.1.6. Preparation of drinking water samples

1 mL drinking water samples were pipetted into 2 mL vial. Using a Hamilton syringe 10 µL aqueous solution of $^{13}C_3$-acrylamide internal standard were added to the vial. This mixture were injected to the gas chromatograph.

4.1.7. Concentrations of the calibration curve solutions, quality assurance of the measurements

For the analyses, 1.0 mg/mL acrylamide and 100 µg/mL D_3-acrylamide stock solutions were prepared sing acetonitrile. Stock solutions were stored at -20 °C protected from light for up to two years. From the stock solutions, working solutions and then calibration solutions were prepared. Two calibration series were prepared for the measurements. The calibration curve was recorded in the 5-1,000 µg/kg range.

In the course of the analyses, standard addition samples were also measured in each measurement sequence, with 3 calibration points per sequence. The current state of calibration was only accepted when the regression value was $r^2 > 0.99$. The acceptance range of the recovery values was 70%–120%. End results were corrected with recovery values within the acceptance range. MRM ion ratios of unknown samples could differ from the MRM ion ratios of the standards, measured with the same parameters, by no more than 20%. Accepted retention times of the target components were within ±2.5% compared to the retention times of the components of the calibration solutions. The quality of the results of food and water samples analyzed between 2009 and 2017 was verified by the repeat analysis of a certified reference material. The calibration curve used for the calibration is shown in ***Figure 11***. The curve was recorded in the 20, 50, 100, 200, 500 and 1000 ng/mL (µg/kg) range. The r^2 value of the fit was 0.9925. Based on the calibration data, the limit of quantification as determined to be LOQ = 10 µg/kg (for water samples the LOQ was 1.0 µg/L). ***Figure 12*** shows the chromatograms obtained when measuring the certified reference material (CRM) with an acrylamide content of 54 µg/kg at the transitions 72→55, 72→54 and 72→44 Da.

4.1.8. Main chromatographic and mass spectrometric parameters

- HPLC column: Hypercarb™ (50 mm × 2.1 mm), with a Hypercarb™ (10 mm × 2.1 mm) precolumn,
- Column oven: 22 °C ± 2 °C,
- Injection volume: 10 µL,
- Mobile phase: 0.1% acetic acid in water,
- Eluent flow rate: 400 µL/min,
- Chromatography time: ca. 8 min (acrylamide eluted at 1.7 min),
- Blow-off gas: 400 °C N_2 600 L/h,
- Nebulizer gas: N_2,
- Cone gas: N_2,
- Collision gas: Ar, with a pressure of 2.3×10^{-3},
- Ion source: positive ESI 125 °C,
- Capillary: 2 kV,
- Cone voltage: 20 V,
- Hexapol voltage: 10 V,
- Typical ion transitions: 72 → 55, 75 → 58 (9 eV), 72 → 44 (20 eV), 72 → 54 (16 eV).

Analyses were carried out with an Agilent TripleQuad 6410/6490 LC-MS/MS instrument in MRM measurement mode.

4.1.9. Main gas chromatographic and mass spectrometric parameters (for water samples)

- Instrument: Agilent 6890N gas chromatograph,
- Carrier gas: helium, constant flow: 1.3 cm^3/min (41 cm/sec),
- Column: Stabilwax (30 m x 0.25 mm x 0.25 µm),
- Temperature program: 30 °C (2 min), 25 °C / min up to 200 °C (3 min),
- Injection: pulsed splitless, 2 µL,
- Injector temperature: 250 °C,
- Mass spectrometer: Agilent 5973N MSD,
- Transfer line temperature: 300 °C,
- Mode of analysis: selective ion monitoring (SIM), m/z = 71 and 74.

5. Analytical results

5.1. Analytical results of water samples

Between the years 2006 and 2017, according to the orders of our customers, 250 water samples of Hungarian origin were analyzed with an LOQ = 1.0 µg/L. Acrylamide levels in all our samples were below the LOQ value.

5.2. Analytical results of solid food samples

Up until the end of 2017, a total of 783 solid food samples were sent to our laboratory for acrylamide determination. Among them were 14 salted biscuit, 28 bakers' wares, 25 other foodstuff and 716 potato chip samples. Analysis of the samples in question were ordered by our customers, even though there were no legally regulated reference values for foodstuffs in the European Union during this period.

The acrylamide contents of the 14 salted biscuits were between the LOQ value of 10 µg/kg and 290 µg/kg. The average acrylamide concentration was 126 µg/kg.

Our laboratory tested 28 bakers' wares. In most of the products, the amount of acrylamide was below the LOQ. There were only 5 samples with values between 30 and 50 µg/kg. It should be noted that, according to the current regulation, the permissible acrylamide level of breads is 50 µg/kg, therefore the investigated samples would have been conform to the new legislation **[9]**.

The remaining 25 different food samples cannot be classified into uniform product groups (seeds, vegetable fats, vegetable products, dairy products, etc.). With the exception of 5 samples, the acrylamide levels of all products were below the LOQ value. Acrylamide concentrations above the LOQ were in the 70-220 µg/kg range. Because of the unique nature of the samples, no meaningful conclusions could be drawn from the analytical results.

The majority of our solid food samples were potato-based products, such as chips, crisps and fried potatoes. Of the 715 products analyzed between 2006 and 2017, acrylamide could not be detected in only 21 of them (2.9% „negative" samples).

This means that 97.1% of the foodstuffs made from potatoes contained acrylamide in amounts exceeding the LOQ value (97.1% „positive" samples). Of the positive samples, 106 contained acrylamide at a level exceeding the 750 µg/kg limit value of the regulation currently in force. This number represented 14.8% of all potato-based products. The concentration values of this product group depicted in ***Figure 13*** in descending order resembles a distribution function. The line drawn across the diagram area indicates the maximum permissible value according to the regulation in force in 2018 (750 µg/kg). ***Figure 14*** shows the histogram of the measurement results of potato-based products.

6. Conclusions

The harmful effect of acrylamide on human health has been recognized by science for decades. The reactive NH_2 group of the molecule reacts readily with vital proteins, including the enzymes responsible for the genetic regulation of cell division. Its presence in the foods has been known since 2002. In the literature on food safety, there are plenty publications on the biochemical, neurotoxic and genotoxic effects of the compound, as well as on methods aimed at detecting its presence with the highest possible sensitivity. With the development of analytical techniques, more and more complex food matrices can be analyzed.

In the 16 years since 2002, based on the results of a thorough assessment, it was suggested by the responsible institutions of the European Union that the Commission adopt a regulation establishing mitigation measures and benchmark levels for the reduction of the presence of acrylamide in foods. Thus, the regulation that entered into force in 2017 came about, the rules of which are mandatory for all catering establishments and production sites that manufacture goods for public consumption.

I welcome the fact that, despite the lack of EU regulation, our customers have had the acrylamide content of their products checked for more than 10 years. Taking into consideration the results of the 1,033 products tested in our laboratory over the past 11 years, meaningful conclusions could be drawn from the acrylamide contents of potato-based products. The statistical distribution of the acrylamide content of the 716 chips, crisps and fried products, despite the fact that our samples did not come from a systematic, representative sampling, showed a good agreement with worldwide literature data surveyed within the limited framework of this publication. 97.7% of our potato-based samples contained measurable amounts of acrylamide, and 14.8% of them would have been objectionable, had the acrylamide contents of the products in question been compared to the limit values that will be effective in the European Union starting from 11, April 2018. Acrylamide is present in many other types of food, and to reduce its amount, effective and responsible measures have to be taken in the future. Based on our analytical results, it seems justified to further monitor the appearance of the products of the Maillard reaction in our foods. Measuring the acrylamide content of foods may be an increasingly important task in the future. To this end, our laboratory continuously improves its professional knowledge and analytical capabilities.

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8. Literature

[1] Billaud, C., Adrian, J. (2003): Louis-Camille Maillard, 1878–1936. Food Reviews International. 19 (4) pp. 345-374.

[2] Thomas, A., Thomas, A. (2012): Acrylamide- A Potent Carcinogen in Food. International Journal of Science and Research. 3 (7) pp. 177-180.

[3] Fennell, T. R., Summer, S. C. J., Snyder, R. W., Burgess, J., Spicer, R., Bridson, W. E., Friedman, M. (2004): Metabolism and Hemoglobin Adduct Formation of Acrylamide in Humans. Toxicological Sciences (85) pp. 447-459.

[4] Ogawa, M., Oyama, T., Isse, T., Yamaguchi, T., Murakami, T., Endo, Y., Kawamoto, T. (2006): Hemoglobin Adducts as a Marker of Exposure to Chemical Substances, Especially PRTR Class I Designated Chemical Substances. Journal of Occupation Health. 48 (5) pp. 314-318.

[5] Tareke, E., Rydberg, P., Karlsson, P., Eriksson, S., Törnquist, M, (2002): Analysis of Acrylamide, a Carcinogen Formed in Heated Foodstuffs. Journal of Agric. Food Chemistry. 50 (17) pp. 4998-5006.

[6] Mc Sweeney, B., Day, C. (Editors) (2002): European Union Risk Assessment Report. Acrylamide. Institute for Health and Consumer Protection, European Chemicals Bureau. European Commission Joint Research Centre. EUR 19835 EN. Volume 24. pp. 1-221.

[7] Mc Sweeney, B., Day, C. (Editors) (2002): European Union Risk Assessment Report. Acrylamide. Institute for Health and Consumer Protection, European Chemicals Bureau. European Commission Joint Research Centre. EUR 19835 EN. Volume 24. p. 66.

[8] Cherrie J. W., Gorman Ng, M., Shafrir, A., van Tongeren, M., Mistry, R., Warwick, O., Corden, C., Rushton, L., Hutchings, S. (2011): Health, Socio-economic and Environmental Aspects of Possible Amendments to the EU Directive on the Protection of Workers from the Risk Related to exposure to Carcinogenes and Mutagenes at Work. Acrylamide. IOM Research Project. P937/9 May 2011. p. 1.

[9] European Commission (2017): Commission Regulation (EU) 2017/2158 of (20.11.2017) Establishing Mitigation Measures and Benchmark Levels for the Reduction of the Presence of Acrylamide in Food. http://eur-lex.europa.eu/legal-content/HU/TXT/?uri=PI_COM:C(2017)7658. (Acquired: 01.02.2018)

[10] Hodge J. E. (1953): Dehydrated Foods, Chemistry of Browning Reactions in Model Systems. Journal of Agric. Food Chem. (1) pp. 928–943.

[11] Molnár P. (1991): Élelmiszerek érzékszervi vizsgálata. Akadémiai Kiadó, Budapest, 1991. pp. 11.

[12] Gasztonyi K., Lásztity R. (Editors) (1992): Élelmiszer-kémia 1. kötet. Maillard reakció. pp. 93-96. Mezőgazda Kiadó, Budapest, 1992.

[13] Zyzak, D. V., Sanders, R. A., Stiojanovic, M., Tallmagde, D. H., Eberhart, B. L., Ewalds, D. K., Gruber, D. C., Morsch, T. R., Strothers, M. A., Rizzi, G. P., Villagran. M. D. (2003): Acrylamide Formation Mechanism in Heated Foods. Journal of Agric. Food Chem. (51) pp. 4782-4787.

[14] Grob, K. (2005): Measures to reduce acrylamide in food. Official Food Control Authority of the Canton of Zurich, Switzerland. Personal PPT Presentation, Symposia at the Animal Health and Food Control Station of County BAZ Miskolc Hungary.

[15] Intersnack (2008): Acrylamide Reduction Workshop April 2008. TOP 1: Overview General Situation & Background. General Situation concerning Acrylamide in Food Especially Savoury Snacks. PDF Presentation. Doetinchem, Hollandia.

[16] Grob, K. (2005): Acrylamide Invitation to a Trip from a Phone Call Via Analysis to Implementation. Official Food Control Authority of the Canton of Zurich, Switzerland. Personal PPT Presentation. Symposia at the Animal Health and Food Control Station of County BAZ Miskolc Hungary.

[17] EFSA CONTAM Panel (2015): Scientific Opinion on Acrylamide in Food. EFSA Journal 2015;13(6):4104, 321 pp. doi:10.2903/j.efsa.2015.4104. http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2015.4104/epdf (Acquired : 2003.01.2018)

[18] Muttucumaru, N., Powers, S. J., Elmore, J. S., Dodson, A., Briddon, A., Mottram, D. S., Halford, N. G. (2017): Acrylamide-forming Potential of Potatoes Grown at Different Locations, and The Ratio of Free Asparagine to Reducing Sugars at Which Free Asparagine Becomes a Limiting Factor for Acrylamide Formation. Food Chem. (220) pp. 76-86.

[19] Weißhaar, R. (2004): Acrylamide in Heated Potatoe Products. Analytics and Formation Routes. Chemisches und Veterinaruntersuchungsamnt Stuttgart. Presentation 14. 01. 2004. Stuttgart (29 dia slides).

[20] Viator C., Muth, K. (2004): Acrylamide: The Next Food Safety Issue? Choices, The Magazine of Food, Farm and Resource Issues. 1st Quarter 2004. pp. 13-17.

[21] Chen, Y-H., Xia, En-Q., Xu, X-R., Ling, W-H., Li, S., Wu, S., Deng, G-F., Zou, Z-F., Zhou, J., Li, H-B. (2012): Evaluation of Acrylamide in Food from China by a LC/MS/MS Method. International Environ. Res. Public Health. 9. (11) pp. 4150-4158.

[22] European Commission – RASFF portal (2018): Subject: Arylamide. Search Result: 20 notifications https://webgate.ec.europa.eu/rasff-window/portal/?event=searchResultList (Aquired: 02.03.2018)

[23] Bleecker M. L. (1998): Toxic peripheral neuropathy. In: Environmental and Occupational Medicine, 3rd edition, W. N. Rom (Editor). New York: Lippincott-Raven Publishers, pp. 697-707, p. 699.

[24] Cloeren, M. (1992): Acrylamide. In: Hazardous Materials Toxicology, Sullivan, J.B. and Krieger, G.R. (Editors). Baltimore: Williams and Wilkens, pp. 940-945.

[25] Parod, R. (1998): Acrylamide. In: Encyclopedia of Toxicology, Vol. 1, Waxler, P. (Editor) New York: Academic Press, pp. 25-26.

[26] Sanchez, M.R. (1998): Dermatologic principles. In: Goldfrank's Toxicology Emergencies, 6th edition, Goldfrank, L.R., Flomenbaum, N.E., Lewin: N.A., Weisman, R.S., Howland, M.A., Hoffman, R.S. (Editors). Connecticut: Appleton and Lange, pp. 474-499.

[27] Sitting, M. (1991): Acrylamide. Handbook of Toxic and Hazardous Chemicals and Carcinogens, 3rd edition Vol. 1, pp. 49-51.

[28] Kiss Zs., Toldi J. (2013): Élelmiszertoxikológia. Szegedi Tudományegyetem, Élettani, Szervezettani és Idegtudományi Tanszék. Jegyzet. pp. 82-83., pp. 91-94.

[29] Neuhäusser-Klaus, A., Schmall, W. (1989): Mutagenic and teratogenic effects of Acrylamide in the Mammalian Spot Test. Mutation Research, 226. pp. 157-162.

[30] Russell, L. B., Hunsicker, P. R., N. L.A., Cacheiro, W. M. Generoso (1990): Induction of Specific-locus Mutations in Male Germ Cells of the Mouse by Acrylamide Monomer. Mutation Research, 262. pp. 101-107.

[31] Zhou, B., Yang, L., Sun, Q., Cong, R., Gu, H., Tang, N., Zhu, H., Wang, B. (2008): Cigarette Smoking and the Risk of Endometrial Cancer: A Meta-Analysis. The American Journal of Medicine 121, pp. 501-508.

[32] Je, Y. (2014): Dietary Acrylamide Intake and Risk of Endometrial Cancer in Prospective Cohort Studies. Arch. Gynecol. Obstet. 291, pp. 1395–1401.

[33] Sciandrello, G., Mauro, M., Caradonna, F., Catanzaro, I., Saverini, M., Barbata, G. (2010): Acrylamide Catalytically Inhibits Topoisomerase II in V79 Cells. Toxicology in Vitro. 24. pp. 830–834.

[34] Thakurela S., Garding, A., Jung, J., Schübeler, D., Burger, L., Tiwari, V. K. (2013): Gene regulation and priming by topoisomerase IIα in embryonic stem cells. Nature Communications | 4:2478 | DOI: 10.1038/ncomms3478 | www.nature.com/naturecommunications (Acquired: 02.01.2018)

[35] Annamalai, T., Cheng, B., Keswani, N., Tse-Dinh, Y-C. (2018): A Fluorescence-Based Assay for Identification of Bacterial Topoisomerase I Poisons. In: Drolet, M. (Editor), DNA Topoisomerases: Methods and Protocols, Methods in Molecular Biology, vol. 1703. pp. 259-268.

[36] European Commission (2007): Commission Recommendation of 3 May 2007 on the monitoring of acrylamide levels in food (2007/331/EC). http://www.epa.oszk.hu/00800/00878/01092/pdf/00330040.pdf (Aquired: 02.03.2018)

[37] European Commission (2010): Commission Recommendation of 2 June 2010 on the monitoring of acrylamide levels in food (2010/307/EU) http://eur-lex.europa.eu/legal-content/HU/TXT/PDF/?uri=CELEX:32010H0307&from=HU (Aquired: 02.03.2018)

[38] European Council (1993): Council Regulation (EEC) No 315/93 of 8 February 1993 laying down Community procedures for contaminants in food. https://publications.europa.eu/en/publication-detail/-/publication/ (Aquired: 20.12.2017)

[39] Liu, J., Zhao, G., Yuan, Y., Chen, F., Hu, X. (2008): Quantitative Analysis of Acrylamide in Tea by Liquid Chromatography Coupled with Electrospray Ionization Tandem Mass Spectrometry. Food Chemistry (108) pp. 760–767.

[40] Ishizuka, M., Fujioka, K., Shibamoto, T. (2008): Analysis of Acrylamide in a Complex Matrix of Polyacrylamide Solutions Treated by Heat and Ultraviolet Light. J. Agric. Food Chem. (56) pp. 6093–6096.

[41] Pugayeva, I., Jaunbergs, J., Bartkevicsa, V. (2014): Development of a Sensitive Method for the Determination of Acrylamide in Coffee Using High Performance Liquid Chromatography Coupled to a Hybrid Quadrupole Orbitrap Mass Spectrometer. Food Additive Contaminant. Part A. Chemical Analytical Control Exposition Risk Assessment. 32 (2) pp. 170-179.

[42] Li, X., Li, J., Cao, P., Liu, Y. (2016): High-efficiency Sample Preparation Approach to Determine Acrylamide Levels in High-fat Foods. Journal of Separation Sci. 39. (15) pp. 2950-2954.

[43] Hu, Q., Xu, X., Li, Z., Zhang, Y., Wang, J., Fu, J. (2014): Detection of Acrylamide in Potato Chips Using a Fluorescent Sensing Method Based on Acrylamide Polymerization-induced Distance Increase Between Quantum Dots. Biosensors and Bioelectronics (54) pp. 64–71

[44] Hoenecke, K.; Gatermann, R,; Harder, W.; Hartig, L. (2004): Analysis of Acrylamide in Different Foodstuffs Using Liquid Chromatography-tandem Mass Spectrometry and Gas Chromatography-tandem Mass Spectrometry. Analytica Chimica Acta (520) pp. 207-215.

[45] Mastovska, K, Lehotay, S. J. (2006): Rapid Sample Preparation Method for LC–MS/MS or GC–MS Analysis of Acrylamide in Various Food Matrices; J. Agric. Food Chem. (54) 19. pp. 7001-7008.

[46] Hungarian Standard (2015): Food Analysis. Determination of Acrylamide in Food by Liquid Chromatography Tandem Mass Spectrometry (LC-ESI-MS/MS). EN 16618:2015.