Acrylamide in Bakery Products

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Acrylamide is a contaminant as defined in Council Regulation (EEC) No 315/93 and as such, it is considered a chemical hazard in the food chain. The toxicity of acrylamide has been acknowledged since 2002, among its toxicological effects on humans being neurotoxicity, genotoxicity, carcinogenicity, and reproductive toxicity. Acrylamide has been classified as carcinogenic in the 2A group, with human exposure leading to progressive degeneration of the peripheral and central nervous systems characterized by cognitive and motor abnormalities. Bakery products (bread, crispbread, cakes, batter, breakfast cereals, biscuits, pies, etc.) are some of the major sources of dietary acrylamide.

Keywords: acrylamide ; toxicity ; public health ; legal regulations ; reduction method ; bakery products

1. Introduction

Bakery products are considered staple foods in many countries due to their content of essential nutrients such as proteins, carbohydrates, fiber and vitamins [1]. In addition to these nutrients, bakery products may contain a number of compounds that are formed in them during heat treatment, such as acrylamide, hydroxymethylfurfural and their derivatives [2][3][4]. Assessing the presence and reducing the level of acrylamide formed in heat-treated foods is a major concern in many countries [5].

Acrylamide (AA) was first synthesized in 1949, and a year later it was used as a flocculating and thickening agent in the synthetic materials industry, for drinking water treatment, in cosmetics, in the pulp and paper industry, in the textile industry, in synthesis, dyes and gels, etc.

Acrylamide or acrylic acid amide is a chemical contaminant that is formed during the technological process of baking, frying or grilling certain foods at temperatures above 120 °C and in low humidity conditions [6][7].

Acrylamide is formed mainly in carbohydrate-rich foods, during the Maillard reaction between reducing carbohydrates (glucose, fructose, etc.) and amino acids (especially asparagine), a reaction responsible for the formation of specific taste and color (browning/ frying) [8][9][10].

Acrylamide is considered to have appeared since the discovery of fire and food cooking by methods of baking, frying and grilling, but then its toxic effects in humans and animals, namely nervous system damage, pre- and post-natal development, negative effects on the male reproductive system, the possibility of cancer and genotoxicity were not known [11][12][13][14][15][16].

The harmful effects of acrylamide on human health were discovered in 2002 by a group of Swedish researchers at the University of Stockholm, together with specialists from the Swedish National Food Administration, who sounded the alarm after finding that the population, through food, ingests a much higher amount of AA than the maximum limit allowed at that time in drinking water [17][18][19].

Nowadays, many researchers representing food safety authorities, academia, and food manufacturers have sought to better understand the kinetics and mechanisms of acrylamide formation, studied the influencing factors, asked themselves questions related to bioavailability and toxicity and finally, are trying to find in a continuous way solutions to minimize its formation in foods. Although nowadays a lot of information has become available, there are still important problems to be solution [1][20].

Factors that influence the content of acrylamide in heat-processed foods such as bakery ones are initial concentration of the precursors, their ratio, flour quality such as flour milling intensity, fermentation conditions, the thermal processing methods (for example, baking, frying, toasting), the processing conditions, such as the temperature, heating time, pH, water content and activity, physical state of the food, additives, etc. [1][4][5][21][22][23][24][25].

In recent years the food industry has proposed mitigation strategies toward reducing levels of acrylamide in its products while maintaining the quality parameters unaffected by the adjusted processing conditions [25][26]. These include modifying the product formulations or processing conditions (lowering pH, baking temperature and time), and adding food ingredients that have been reported to inhibit acrylamide formation (organic acids, mainly citric, calcium or magnesium ions, and extracts with antioxidant properties) [2][13]. Researchers have also found other solutions to lower acrylamide levels in foods: to breed wheat genotypes that have low levels of free asparagine concentration [27], such as using the enzyme asparaginase to hydrolyze asparagine to aspartic acid and ammonia prior to cooking or processing (to reduce acrylamide levels by 70–90% without affecting the organoleptic properties of the products) [25][28][29][30]; application of modern processes during the baking process such as radio frequency, inert atmosphere, steam baking, microwaves baking, etc. [4][31][32]; applying glycine and glutamine to dough prior to fermentation [28][33][34]; the use of bacteriocin like inhibitory substances producing lactic acid bacteria (LAB) with high proteolytic activity to perform the fermentation processes with yeast [1][29]; replacing reducing sugars with sucrose [34]; using the different additives, such as rosemary, amino acids or proteins [23][35] etc (Figure 1).

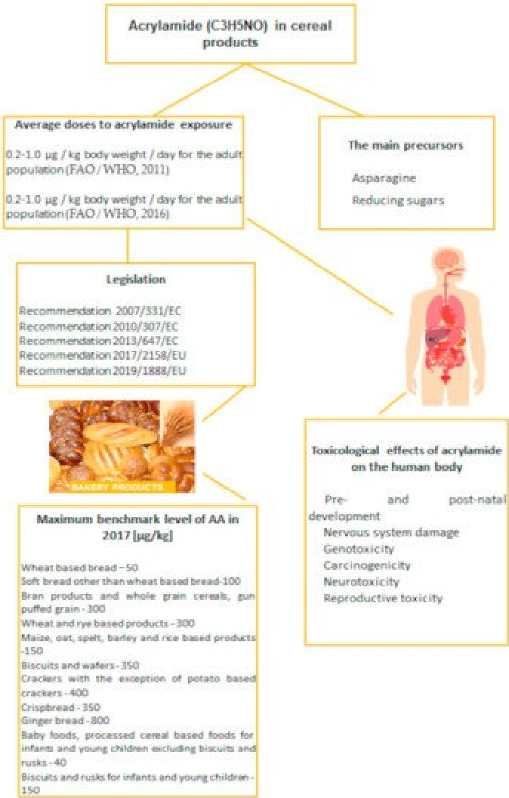

Figure 1. Overview of acrylamide, its toxicity and legal regulations in bakery industry.

2. Toxicological Effects of Acrylamide on the Human Body and Its Risks Represented by It Consumption

Research has shown that AA is absorbed by humans and animals through ingestion, inhalation and skin. After inhalation AA is rapidly distributed to all organs of the body through the bloodstream. AA can be identified in the brain, heart, liver, kidneys and breast milk [36][37].

In the body, AA is metabolized to a chemical, reactive epoxide, glycidamide, following the reaction catalyzed by the cytochrome enzyme [38]. AA conversion to the reactive, mutagenic and genotoxic compound, glycidamide, can occur in both rodents and humans [39]. Glycidamide formation is considered responsible for the genotoxic effects of AA having the potential to induce mutagenic genes at the chromosomal level [40][41].

AA and glycidamide can react with macromolecules, such as hemoglobin, DNA, serum albumin and enzymes, to form adducts. The formation of adducts with DNA is likely to lead to the toxic, carcinogenic potential of AA [42].

Research on laboratory animals has shown that exposure to AA have the following effects: genotoxic, carcinogenic, neurotoxic, affects the male reproductive system and has effects on pre- and postnatal development. AA has contributed to genetic mutations and tumors in various organs. Based on animal experiments, the European Food Safety Authority (EFSA) experts have concluded that AA in food may increase the risk of cancer in consumers of all ages, including children, which is the most exposed age group [43][44][45].

The possible carcinogenic effects of AA have been discovered since 1994 when the International Agency for Research on Cancer (IARC) classified AA as having "carcinogenic potential in humans" (group 2A) [46][47][48].

In April 2002, the Swedish National Food Administration (SNFA) reported that AA is also found in food and that by eating common foods such as bread, biscuits, chips, coffee, French fries, people ingest AA in unknown amounts that can have negative effects on human health [19][49][50][51][52][53].

Later in the same year, 2002, the Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) organized a consultation to gather the views of an international group of experts on the health implications of AA in food. The aim was to review and evaluate new and existing AA data and research, identify needs for further information and studies and also to develop and suggest possible interim advice for governments, industry and consumers [45][54].

In 2005, the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2005) conducted an evaluation of available data on AA. The full report was published in 2006. Food intakes were estimated to be between 1 µg/kg (medium exposure dose) and 4 µg/kg (high exposure dose) in body weight/day for the general population and consumers with a high intake. These estimates also included children [46][55].

JECFA concluded that epidemiological studies and data on biomarkers available in humans and animals at the time of assessment were not adequate to establish a dose-response relationship and therefore performed the assessment based on available animal studies. JECFA considered the genotoxicity and carcinogenicity of AA to be essential effects for risk assessment, but also considered other non-cancerous endpoints of concern, such as effects on the nervous system. JECFA concluded that, at an estimated average consumption, morphological changes of the nerves could not be excluded for some individuals [45][55].

JECFA recommended continuing efforts to reduce AA concentrations in food and that AA be revaluated when the results of long-term carcinogenicity and neurotoxicity studies become available [45][56].

In April 2005, the EFSA Scientific Panel on Contaminants in the Food Chain (CONTAM Group) agreed with the main conclusions and recommendations of JECFA on AA risk assessment. The CONTAM Group noted the use of data from European countries, including information collected in collaboration initiatives between the European Commission and EFSA, and concluded that further evaluation by EFSA was not necessary at that time [31][45].

In 2005, the Panel of Experts of the National Centre for Toxicology for the Risk Assessment of Human Reproduction (NTP-CERHR) published a report on the toxicity of AA reproduction and development [57]. The report concluded that there are no human data available on the developmental or reproductive toxicity of AA and that the available experimental data were sufficient to conclude that AA may cause developmental toxicity in rats and mice. The NTP-CERHR expert group also concluded that there is sufficient data to conclude that AA induces transmissible genetic lesions in male germ cells of mice in the form of mutual translocations and genetic mutations.

In 2008, EFSA organized a scientific colloquium on AA carcinogenicity and new evidence related to dietary exposure [58][59][60]. The aim of this EFSA colloquium was to discuss the challenges regarding the potential toxicity and cancer risk associated with food exposure to AA, given the new information that has become available since the last risk assessment carried out by JECFA in 2005 [55].

In 2011, JECFA the researchers concluded that the average dose of exposure to AA is 0.2–1.0 µg/kg body weight/day for the adult population, while 95% of cases were in the range of 0.6–1.8 µg/kg body weight/day [61].

In 2015, the Scientific Group for Food Chain Contaminants (CONTAM) of the European Food Safety Authority adopted an opinion on AA in food. Based on animal studies, the Authority confirms previous assessments that food acrylamide may increase the risk of developing cancer for consumers in all age groups [62]. Given that acrylamide is present in a wide range of foods consumed daily, this warning applies to all consumers, but children are the most exposed age group based on body weight. The possible harmful effects of acrylamide on the nervous system, on prenatal and postnatal development and on male reproduction were not considered to be a cause for concern, based on current levels of dietary exposure. Current levels of dietary exposure to acrylamide in all age groups indicate a concern about its carcinogenic effects [32][45][63][64].

3. Legislative Rules on the Maximum Benchmark Levels of Acrylamide in

Bakery Products

Depending on the culinary habits of various countries, the intake of AA in the daily diet can vary between 10–30% for bakery products and 10–20% for pastries. Thus, in Germany, bread and rolls represent approximate 25% of the daily intake of AA, whereas in the Netherlands and Belgium, bread represents 10% of the daily intake of AA, and in Sweden up to 17%. In Romania, bread contributes between 14–37.5% of the maximum allowed dose of AA for food in the daily diet, estimated by FAO/WHO [60].

Thus we can consider that among the foods with the highest intake of AA in the daily diet are bread and bakery products [65]. Some researchers have reported that the highest level of AA is found in the crust of bread and less in bread crumbs [66].

Since 2005 EFSA, through the Scientific Committee for Contaminants in the Food Chain, recognizes the presence of AA in food and together with food industry operators, specialists and researchers from member countries have researched AA training pathways and developed a set of measures to reduce the level of this contaminant in food.

Thus, in 2007, the European Commission issued 2007/331/EC, on monitoring the level of AA in food which provides for a three-year monitoring program (2007–2009) of AA in certain foods, in order to provide concrete information on foodstuffs that have a high level of acrylamide or those that by consumption high food have a significant contribution to its assimilation. The categories of bakery products covered by the monitoring program are: bread; breakfast cereals; biscuits, including infant biscuits; baby food preserved in glass containers which may or not contain cereals; processed baby food based on cereals; other products (containing cereal products and baby food, other than those mentioned above).

The Recommendation of the European Commission (EC) 2007/331/EC [67] requires that sampling by member states to be carried out in both production and marketing units (e.g., supermarkets, smaller shops, bakeries, restaurants, etc.). A minimum number of samples were required for each product category, for each country, and a unitary model for reporting the types of food taken and the results obtained. A total number of 2042 samples/year were established, and Romania planned a number of 80 samples, with eight annual samples for each category of food product, respectively. During the 2017–2019 periods in Romania the National Sanitary Veterinary and Food Safety Authority have been analyzed a total of 138 samples from which 50 were analyzed in 2018 and 88 in 2019. No foods samples have been analyzed in 2017. However, in a complementary way the National Research & Development Institute for Food Bioresources, IBA Bucharest have been analyzed AA level from various foods products from Romania market. A number of 55 cereal products were evaluated in Romania during 2017–2018 periods [54][59].

Worldwide, in recent years many new methods have been developed for the determination of AA in food products, such as: high performance liquid chromatography mass spectrometry (HPLC–MS), high performance liquid chromatography tandem mass spectrometry (HPLC–MS/MS), gas chromatography-electron capture detection (GCECD), gas chromatography-mass spectrometry (GC-MS), liquid chromatography (LC) with ultraviolet (UV), or MS detection, and capillary electrophoresis (CE) with UV and MS, [22][24][56][68][69][70], solid-phase extraction (SPE) (were used to analyze acrylamide in bread), and dispersive liquid-liquid microextraction (DLLME) [71]. The ultra-performance liquid chromatography (UPLC) method has the advantage that the resolution is improved with higher analytical sensitivity and a shorter retention time [72]. The European Committee for Standardization published a method for AA detection in bakery products through liquid chromatography tandem mass spectrometry (LC-ESI-MS/MS) described in EN standard 16618:2015. In 2017 the European Committee for Standardization (CEN) published a technical specification for AA detection through gas chromatography (GC) coupled with mass spectrometry (MS) method in FprCEN/TS 17083 [73]. In Romania, at the national authority level, the laboratory accredited to perform AA determination uses high performance liquid chromatography coupled with a diode array detector (HPLC-UV). The separation of AA is done by passing on the chromatographic column followed by identification, following the series of photodiodes of the diode array detector (DAD) detector and identification by comparing TR (retention times) with TR of a standard substance. Also the AA confirmation is made by analyzing the spectrum obtained on the DAD detector. The interpretation of the results consists in identifying the compounds of interest by comparing the retention times of the peaks in the recorded chromatograms and by analyzing the DAD spectrum for the samples to be analyzed with the retention times of the peaks in the chromatograms recorded for the standard substance. The method present a good sensitivity: LOQ and LOD were 50 and 20 μg kg^{-1} for potato, coffee and potatoes products and 25 and 20 μg kg^{-1} for cereals, bread and bakery products respectively. All the methods used for AA determination are time-consuming and expensive. Therefore, nowadays specialists are trying to find rapid and low costs solutions for AA determinations. One of the quick methods for assessing the AA content from bakery products is to correlate some color values such as browning and HMF content with AA value. This method is only a predictive one and applicable only for heat products such as bakery ones [31].

The sampling method chosen by the Member States, in order to ensure the representativeness of the samples for the batch to be sampled, must follow the sampling procedures provided for in Regulation 2007/1234/EC. Also, the regulation 2007/333/EC is presenting also the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs [74][75]. The reporting of the values obtained by AA is done taking into account the limit of quantification (LOQ), and to guarantee the comparability of the results, the analysis methods must be chosen so that the LOQ is 30 µg/kg for bread and bakery products. If, repeatedly, for the same product type, the AA value is below the LOQ, it will be replaced with another product [76].

In the case of bakery products, the specific information to be provided is: type of bread, soft or crunchy, fiber content, type of cereals, fermented or unfermented, type of fermentation (e.g., yeast), and other ingredients. The choice of the type of bread sampled must reflect the eating habits of each country [5].

In 2009, EFSA published in the scientific report entitled "*Results on the monitoring of acrylamide levels in food*" the results for 2007 and concluded that the measures to reduce the level of acrylamide were not constantly applied by food industry operators (OIA), and the toolbox developed by Food Drink Europe to support economic operators has not achieved the desired effects [77][78].

At the same time, it was found that an improvement of the monitoring program is necessary, regarding the food classification, and thus in 2010, the European Commission publishes the Recommendation 2010/307/EC on monitoring the level of acrylamide in food [79].

With this recommendation, ESFA considers it important to continue collecting data for annual monitoring of AA levels, and for food business operators and Member State authorities to continue research into ways to form acrylamide and how to reduce it.

The novelty of this recommendation is that in order to identify the evolution over time, it is important that products with the same specifications (e.g., the same type of bread) be sampled, if possible, every year. Specific information to be provided for bakery products: type of bread, e.g., wheat, rye, multigrain bread, bread with other ingredients, etc.

Following the two recommendations of the European Union, the Recommendation 2007/331/EC and the Recommendation 2010/307/EC which required the monitoring of acrylamide levels in certain foods during 2007–2010, the European Food Safety Authority (EFSA) published, in 2012, the results of monitoring in the scientific report entitled "Update on acrylamide levels in food from monitoring years 2007–2010" [77]. The report concluded that there was no steady trend in food groups towards lower acrylamide levels and that a decrease in acrylamide levels was observed only in certain food categories, while in other food categories, an increase in these levels was observed.

Thus, in 2013, the European Commission published the Recommendation 2013/647/EC, regarding the analysis of acrylamide levels in foods in which the indicative values for acrylamide are presented, based on EFSA monitoring data for the period 2007–2012 [80].

As AA levels are much higher in certain foods than in similar products in the same product category, the European Commission has recommended that surveys be carried out by the competent authorities of the member states to examine the production and processing methods used by OIA. These surveys will be conducted in case of exceeding the AA level above the indicative values established in this recommendation.

These investigations aim at analyzing the risks and critical control points, the HACCP system of the food business operator, to verify that the relevant technological steps in which acrylamide can form have been correctly identified and that appropriate measures have been taken to reduce them. The indicative values, set out in this Recommendation, do not constitute safety thresholds.

Table 1 shows the indicative values for acrylamide, based on EFSA monitoring data for the period 2007–2012, according to Recommendation 2013/647/EU (European Commission, 2013) and 2018–2021 period according to Recommendation 2017/2158/EU (European Commission, 2017) [80][81].

Table 1. Indicative values for acrylamide in bakery products set by the European Commission for 2007–2021 periods (2013/647/EU; 2017/2158/EU).

Food	Benchmark Level 2013 [μg/kg]	Benchmark Level 2017 [μg/kg]
Soft bread		
Wheat based bread	80	50
Soft bread other than wheat based bread	150	100
Breakfast cereals (excl. porridge)		
Bran products and whole grain cereals, gun puffed grain	400	300
Wheat and rye based products	300	300
Maize, oat, spelt, barley and rice based products	200	150
Biscuits and wafers	500	350
Crackers with the exception of potato based crackers	500	400
Crispbread	450	350
Ginger bread	1000	800
Baby foods, processed cereal based foods for infants and young children excluding biscuits and rusks	50	40
Biscuits and rusks for infants and young children	200	150

In 2017 the European Commission publishes Regulation 2017/2158/EC—establishing mitigation measures and reference levels to reduce the presence of acrylamide in food [81].

The regulation lays down measures to reduce the levels of acrylamide to be taken by food business operators, if the raw materials contain its precursors, so that these levels are below the reference levels set by the regulation.

The regulation applies to food business operators who produce and place on the market the following bakery products: bread and fine bakery products, breakfast cereals, baby food and food products (which may contain cereals), processed from cereals for small children.

Food business operators covered by this Regulation must identify, depending on the type and nature of their activities, the stages of food processing that are susceptible to the formation of acrylamide and determine, in the context of risk analysis and in accordance with the recommendations of this Regulation, appropriate measures to reduce acrylamide levels.

Diminution measures, in the case of producers who distribute products at county, national, or global level, should be included by the OIA in the procedures of the risk analysis system and critical control points (HACCP) of the production unit, or in the procedures good hygiene practices. The effectiveness of mitigation measures should be verified by sampling and analysis, for which analytical requirements and sampling frequency should be established, to ensure that the analytical results obtained are representative. This requirement does not apply to OIAs that produce food locally and perform only local retail activities. The values obtained for AA must be below the reference levels established by this Regulation. In order to verify the correct implementation of the measures to reduce the level of AA and to carry out the correct sampling and analysis, official controls will be performed by the authorities of the Member States [82].

Within this regulation, the reference levels have been revised, which are performance indicators, which aim to verify the correctness of the application of the reduction measures. These reference values shall be set at a very low level and shall be periodically reviewed every three years in accordance with this Regulation so as to ensure that the level of acrylamide is reduced to a very low level [82].

It is maintained the recommendation that the sampling and analysis of AA be performed according to Regulation 2007/333/EC (European Commission, 2007) presenting the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo (a) pyrene in foodstuffs [75].

The reference levels shown in Table 1 for detecting the presence of acrylamide in bakery products, according to 2017/2158/EC (European Commission, 2017) laying down reduction measures and reference levels for reducing the presence of acrylamide in foodstuffs.

Because it was concluded that Regulation 2017/2158/EC did not present sufficient available data on the presence of acrylamide in foods [81] was adopted in 2019 by the European Commission the Recommendation 2019/1888/EC—on monitoring the presence of acrylamide in certain foods including bakery products [83]. This normative act adds a new list of non-exhaustive food products, which must be monitored in order to identify the risks and adopt new prevention and/or reduction measures against this contaminant.

The new food list adds the following specialties for bakery products: buns (hamburger buns, whole wheat buns and milk buns, etc.); sticks, Mexican tortillas; horns; doughnuts; bread specialties (e.g., pumpernickel bread, olive ciabatta, onion bread, etc.); pancakes; crispy cookies made of a thin layer of dough and fried in oil; churros.

The obligation to monitor AA levels and the effectiveness of measures to reduce it, as well as that of Member States and OIA to transmit to EFSA, the data collected in the previous year, in order to compile a database, remains.

The recommendation is maintained that the sampling and analysis of AA be performed according to Regulation 2007/333/EC (European Commission, 2017) laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs.

By adopting the Recommendation 2019/1888/EC (European Commission, 2019), the Recommendations 2010/307/EC (European Commission, 2010) and 2013/647/EC (European Commission, 2013) are repealed [79][83][84].

4. Methods to Reduce the Acrylamide Content in Bakery Products

In 2005 the Food Drink Europe Organization, who represents economic operators in the EU food and beverage industry, developed a set of “acrylamide toolbox” tools, which includes the most complete information from authorities, scientists, international research organizations and economic operators, about the ways of forming AA and the methods of reducing it from certain food groups. This “toolkit” is revised periodically, the 15th version being published in 2019. This material presents 14 different parameters (“tools”), grouped into four broad categories (“toolkit compartments”): agronomic factors, manufacturing recipe, food processing method and final preparation. These “tools” can be used selectively by the OIA, depending on the specific needs of each, in order to reduce the level of acrylamide in their products (EFSA, 2007, Food Drink Europe, 2019) [85][86].

According to the Regulation 2017/2158/EC (European Comission, 2017), the operators in the food industry are obliged to apply measures to reduce the level of AA, so as to reach the lowest possible levels, below the reference levels established in this normative act [81].

The mitigation measures must be adapted according to the nature of the activity of the operators in the field and include measures both in the agricultural sector and during the technological process of preparation of bread and bakery products [87].

In 2009 Codex Alimentarius drew up a guide "Code of practice for the reduction of acrylamide in foods" -CAC/RCP 67-2009, which specifies measures to reduce AA levels. Food business operators are advised to test in their own units all the measures recommended in this guide, in order to identify the optimal method to reduce this contaminant [88].

For the application of measures to reduce the level of AA in food, it must be taken into account that these methods do not affect the organoleptic and microbiological properties of finished products, their nutritional qualities and the associated consumer acceptability [88].

Worldwide there are three groups of strategies to reduce acrylamide formation: (i) modification of raw materials, (ii) optimization of processing conditions, and (iii) addition of exogenous additives [89].

If we compile all the mitigation measures recommended in the "toolkit" of EuropeDrinkFood, in the Guide developed by Codex Alimentarius, as well as those specified in the Regulation 2017/2158/EC (European Commission, 2017) [81], we find that they must be applied starting with the cereals cultivation stage and continuing throughout the technological process until the elaboration of baking instructions in the case of products intended to be completed at home or in public catering units. It has been established that AA formation in different bread types is influenced by the presence of free asparagine and reducing sugars that are associated with cultivar and crop's type storage condition and harvest season [90].

References

1. Nachi, I.; Fhoula, I.; Smida, I.; Taher, I.B.; Chouaibi, M.; Jaunbergs, J.; Hassouna, M. Assessment of lactic acid bacteria application for the reduction of acrylamide formation in bread. LWT 2018, 92, 435–441.
2. Miśkiewicz, K.; Nebesny, E.; Rosicka-Kaczmarek, J.; Żyżelewicz, D.; Budryn, G. The effects of baking conditions on acrylamide content in shortcrust cookies with added freeze-dried aqueous rosemary extract. J. Food Sci. Technol. 2018, 55, 4184–4196.
3. Miśkiewicz, K.; Rosicka-Kaczmarek, J.; Nebesny, E. Effects of chickpea protein on carbohydrate reactivity in acrylamide formation in low humidity model systems. Foods 2020, 9, 167.
4. Mogol, B.A.; Gökmen, V. Mitigation of acrylamide and hydroxymethylfurfural in biscuits using a combined partial conventional baking and vacuum post-baking process: Preliminary study at the lab scale. Innov. Food Sci. Emerg. Technol. 2014, 26, 265–270.
5. Keramat, J.; LeBail, A.; Prost, C.; Jafari, M. Acrylamide in baking products: A review article. Food Bioprocess Technol. 2011, 4, 530–543.
6. Nematollahi, A.; Kamankesh, M.; Hosseini, H.; Ghasemi, J.; Hosseini-Esfahani, F.; Mohammadi, A.; Mousavi Khaneghah, A. Acrylamide content of collected food products from Tehran's market: A risk assessment study. Environ. Sci. Pollut. Res. 2020, 27, 30558–30570.
7. Abt, E.; Robin, L.P.; McGrath, S.; Srinivasan, J.; DiNovi, M.; Adachi, Y.; Chirtel, S. Acrylamide levels and dietary exposure from foods in the United States, an update based on 2011–2015 data. Food Addit. Contam. Part A 2019, 36, 1475–1490.
8. Mesías, M.; Morales, F.J.; Delgado-Andrade, C. Acrylamide in biscuits commercialised in Spain: A view of the Spanish market from 2007 to 2019. Food Funct. 2019, 10, 6624–6632.
9. Andačić, I.M.; Tot, A.; Ivešić, M.; Krivohlavek, A.; Thirumdas, R.; Barba, F.J.; Brnčić, S.R. Exposure of the Croatian adult population to acrylamide through bread and bakery products. Food Chem. 2020, 322, 126771.
10. Mousavi Khaneghah, A.; Fakhri, Y.; Nematollahi, A.; Seilani, F.; Vasseghian, Y. The concentration of acrylamide in different food products: A global systematic review, meta-analysis, and meta-regression. Food Rev. Int. 2020, 2–19.
11. Crawford, L.M.; Kahlon, T.S.; Chiu, M.C.M.; Wang, S.C.; Friedman, M. Acrylamide content of experimental and commercial flatbreads. J. Food Sci. 2019, 84, 659–666.
12. Salimi, A.; Pashaei, R.; Bohlooli, S.; Vaghar-Moussavi, M.; Pourahmad, J. Analysis of the acrylamide in breads and evaluation of mitochondrial/lysosomal protective agents to reduce its toxicity in vitro model. Toxin Rev. 2020, 1–10.
13. Schouten, M.A.; Tappi, S.; Romani, S. Acrylamide in coffee: Formation and possible mitigation strategies—A review. Crit. Rev. Food Sci. Nutr. 2020, 60, 3807–3821.
14. Schouten, M.A.; Genovese, J.; Tappi, S.; Di Francesco, A.; Baraldi, E.; Cortese, M.; Romani, S. Effect of innovative pre-treatments on the mitigation of acrylamide formation in potato chips. Innov. Food Sci. Emerg. Technol. 2020, 64, 102397.
15. Žilić, S. Acrylamide formation in biscuits made of different wholegrain flours depending on their free asparagine content and baking conditions. Food Res. Int. 2020, 132, 109109.
16. Cantrell, M.S.; McDougal, O.M. Biomedical rationale for acrylamide regulation and methods of detection. Compr. Rev. Food Sci. Food Saf. 2021, 20, 2176–2205.
17. Jeong, H.; Hwang, S.; Kwon, H. Survey for acrylamide in processed foods from Korean market and individual exposure estimation using a non-parametric probabilistic model. Food Addit. Contam. Part A 2020, 37, 916–930.

18. Koszucka, A.; Nowak, A.; Nowak, I.; Motyl, I. Acrylamide in human diet, its metabolism, toxicity, inactivation and the ass ociated European Union legal regulations in food industry. Crit. Rev. Food Sci. Nutr. 2020, 60, 1677–1692.

19. Rifai, L.; Saleh, F.A. A review on acrylamide in food: Occurrence, toxicity, and mitigation strategies. Int. J. Toxicol. 2020, 39, 93–102.

20. Mustafa, A.; Kamal-Eldin, A.; Petersson, E.; Andersson, R.; Åman, P. Effect of extraction pH on acrylamide content in fr esh and stored rye crisp bread. J. Food Compos. Anal. 2008, 21, 351–355.

21. Wang, S.; Yu, J.; Xin, Q.; Wang, S.; Copeland, L. Effects of starch damage and yeast fermentation on acrylamide forma tion in bread. Food Control 2017, 73, 230–236.

22. Gerssena, A.; Boveea, T.; van Ginkela, L.; van Ierselb, M.; Hoogenbooma, R. Food and feed safety: Cases and approa ches to identify the responsible toxins and toxicants. Food Control 2019, 98, 9–18.

23. Vingborg Hedegaard, R.; Granby, K.; Frandsen, H.; Thygesen, J.; Skibsted, L. Acrylamide in bread. Effect of prooxidant s and antioxidants. Eur. Food Res. Technol. 2008, 227, 519–525.

24. Zhuang, H.; Zhang, T.; Liu, J.; Yuan, Y. Detection of acrylamide content in traditional Chinese food by high-performance liquid chromatography tandem mass spectrometry method. CyTA J. Food 2012, 10, 36–41.

25. Curtis, T.; Halford, N. Reducing the acrylamide-forming potential of wheat. Food Energy Secur. 2016, 5, 153–164.

26. Gökmen, V.; Açar, Ö.C.; Köksel, H.; Acar, J. Effects of dough formula and baking conditions on acrylamide and hydroxy methylfurfural formation in cookies. Food Chem. 2007, 104, 1136–1142.

27. Ohm, J.B.; Simsek, S.; Mergoum, M. Variation of protein MWD parameters and their associations with free asparagine concentration and quality characteristics in hard red spring wheat. J. Cereal Sci. 2018, 79, 154–159.

28. Huang, W.; Yu, S.; Zou, Q.; Tilley, M. Effects of frying conditions and yeast fermentation on the acrylamide content in yo u-tiao, a traditional Chinese, fried, twisted dough-roll. Food Res. Int. 2008, 41, 918–923.

29. Bartkiene, E.; Jakobsone, I.; Juodeikiene, G.; Vidmantiene, D.; Pugajeva, I.; Bartkevics, V. Study on the reduction of ac rylamide in mixed rye bread by fermentation with bacteriocin-like inhibitory substances producing lactic acid bacteria in combination with Aspergillus niger glucoamylase. Food Control 2013, 30, 35–40.

30. Anese, M.; Quarta, B.; Peloux, L.; Calligaris, S. Effect of formulation on the capacity of l-asparaginase to minimize acryl amide formation in short dough biscuits. Food Res. Int. 2011, 44, 2837–2842.

31. Michalak, J.; Czarnowska-Kujawska, M.; Klepacka, J.; Gujska, E. Effect of Microwave Heating on the Acrylamide Form ation in Foods. Molecules 2020, 25, 4140.

32. Gülcan, Ü.; Uslu, C.C.; Mutlu, C.; Arslan-Tontul, S.; Erbaş, M. Impact of inert and inhibitor baking atmosphere on HMF and acrylamide formation in bread. Food Chem. 2020, 332, 127434.

33. Claus, A.; Weisz, G.M.; Kammerer, D.R.; Carle, R.; Schieber, A. A method for the determination of acrylamide in bakery products using ion trap LC-ESI-MS/MS. Mol. Nutr. Food Res. 2005, 49, 918–925.

34. Mustafa, A.; Fink, M.; Kamal-Eldin, A.; Rosén, J.; Andersson, R.; Åman, P. Interaction effects of fermentation time and a dded asparagine and glycine on acrylamide content in yeast-leavened bread. Food Chem. 2009, 112, 767–774.

35. Bråthen, E.; Knutsen, S.H. Effect of temperature and time on the formation of acrylamide in starch-based and cereal m odel systems, flat breads and bread. Food Chem. 2005, 92, 693–700.

36. Matoso, V.; Bargi-Souza, P.; Ivanski, F.; Romano, M.A.; Romano, R.M. Acrylamide: A review about its toxic effects in th e light of Developmental Origin of Health and Disease (DOHaD) concept. Food Chem. 2019, 283, 422–430.

37. Mollakhalili-Meybodi, N.; Khorshidian, N.; Nematollahi, A.; Arab, M. Acrylamide in bread: A review on formation, health r isk assessment, and determination by analytical techniques. Environ. Sci. Pollut. Res. 2021, 28, 15627–15645.

38. Pundir, C.S.; Yadav, N.; Chhillar, A.K. Occurrence, synthesis, toxicity and detection methods for acrylamide determinati on in processed foods with special reference to biosensors: A review. Trends Food Sci. Technol. 2019, 85, 211–225.

39. Sazesh, B. Quinoa as a wheat substitute to improve the textural properties and minimize the carcinogenic acrylamide c ontent of the biscuit. J. Food Process. Preserv. 2020, 44, e14563.

40. Duke, T.J.; Ruestow, P.S.; Marsh, G.M. The influence of demographic, physical, behavioral, and dietary factors on hem oglobin adducts levels of acrylamide and glycidamide in the general US population. Crit. Rev. Food Sci. Nutr. 2018, 58, 700–710.

41. Nematollahi, A.; Kamankesh, M.; Hosseini, H.; Hadian, Z.; Ghasemi, J.; Mohammadi, A. Investigation and determinatio n of acrylamide in 24 types of roasted nuts and seeds using microextraction method coupled with gas chromatography –mass spectrometry: Central composite design. J. Food Meas. Charact. 2020, 14, 1249–1260.

42. Crawford, L.M.; Kahlon, T.S.; Wang, S.C.; Friedman, M. Acrylamide Content of Experimental Flatbreads Prepared from Potato, Quinoa, and Wheat Flours with Added Fruit and Vegetable Peels and Mushroom Powders. Foods 2019, 8, 228.
43. Bignardi, C.; Cavazza, A.; Grimaldi, M.; Laganà, C.; Manzi, C.; Rinaldi, M.; Corradini, C. Acrylamide determination in baked potatoes by HPLC–MS: Effect of steam and correlation with colour indices. Eur. Food Res. Technol. 2019, 245, 2393–2400.
44. Crawford, L.M.; Wang, S.C. Comparative study of four analytical methods for the routine determination of acrylamide in black ripe olives. J. Agric. Food Chem. 2019, 67, 12633–12641.
45. EFSA Panel on Contaminants in the Food Chain (CONTAM). Scientific opinion on acrylamide in food. EFSA J. 2015, 13, 4104.
46. Kafouris, D.; Stavroulakis, G.; Christofidou, M.; Iakovou, X.; Christou, E.; Paikousis, L.; Yiannopoulos, S. Determination of acrylamide in food using a UPLC–MS/MS method: Results of the official control and dietary exposure assessment in Cyprus. Food Addit. Contam. Part A 2018, 35, 1928–1939.
47. Roszko, M.Ł.; Szczepańska, M.; Szymczyk, K.; Rzepkowska, M. Dietary risk evaluation of acrylamide intake with bread in Poland, determined by two comparable cleanup procedures. Food Addit. Contam. Part B 2020, 13, 1–9.
48. Esposito, F.; Velotto, S.; Rea, T.; Stasi, T.; Cirillo, T. Occurrence of Acrylamide in Italian Baked Products and Dietary Exposure Assessment. Molecules 2020, 25, 4156.
49. Khezerolou, A.; Alizadeh-Sani, M.; Zolfaghari Firouzsalari, N.; Ehsani, A. Formation, properties, and reduction methods of acrylamide in foods: A review study. J. Nutr. Fasting Health 2018, 6, 52–59.
50. Kopanska, M.; Muchacka, R.; Czech, J.; Batoryna, M.; Formicki, G. Acrylamide toxicity and cholinergic nervous system. J. Physiol. Pharmacol. 2018, 69, 847–858.
51. Lambert, M.; Inthavong, C.; Hommet, F.; Leblanc, J.C.; Hulin, M.; Guérin, T. Levels of acrylamide in foods included in 'the first French total diet study on infants and toddlers'. Food Chem. 2018, 240, 997–1004.
52. Sawicka, B.; Mohammed, A.; Umachandran, K. Food safety of potato processed in the aspect of acrylamide risk. MOJ Food Process Technol. 2018, 6, 96–102.
53. Stockmann, F.; Weber, E.A.; Schreiter, P.; Merkt, N.; Claupein, W.; Graeff-Hönninger, S. Impact of nitrogen and sulfur supply on the potential of acrylamide formation in organically and conventionally grown winter wheat. Agron. J. 2018, 8, 284.
54. Sarion, C.; Dabija, A.; Oroian, M.; Negoiță, M.; Codină, G.G. Evaluation of acrylamide levels in cereal products from the Romanian market during the 2017 and 2018 period. EuroBiotech J. 2020, 4, 127–133.
55. JECFA 2005. Joint FAO/WHO Expert Committee on Food Additives. Available online: (accessed on 18 March 2021).
56. Negoiță, M.; Culețu, A. Application of an Accurate and Validated Method for Identification and Quantification of Acrylamide in Bread, Biscuits and Other Bakery Products Using GC-MS/MS System. J. Braz. Chem. Soc. 2016, 27, 1782–1791.
57. Manson, J.; Brabec, M.J.; Buelke-Sam, J.; Carlson, G.P.; Chapin, R.E.; Favor, J.B.; Fischer, L.J.; Hattis, D.; Lees, P.S.; Perreault-Darney, S.; et al. NTP-CERHR expert panel report on the reproductive and developmental toxicity of acrylamide. Birth Defects Res. B Dev. Reprod. Toxicol. 2005, 74, 17–113.
58. Claeys, W.; De Meulenaer, B.; Huyghebaert, A.; Scippo, M.-L.; Hoet, P.; Matthys, C. Reassessment of the acrylamide risk: Belgium as a case-study. Food Control 2016, 59, 628–635.
59. Sarion, C.; Dabija, A.; Codină, G.G. Assessment of acrylamide content in some foodstuffs on the Romania market. Sci. Bull. Ser. F Biotechnol. 2020, 24, 62–68.
60. Negoiță, M.; Adascalului, A.; Iorga, E.; Catana, L.; Catana, M.; Belc, N. Internal validation of the method for determination of acrylamide in bread by gas chromatography tandem mass spectrometry. Rev. Chim. 2015, 66, 464–471.
61. JECFA. Evaluation of Certain Food Additives and Contaminants. 72nd Report of the Joint FAO/Who Expert Committee on Food Additive; WHO Press: Geneva, Switzerland, 2011.
62. Jouquand, C.; Niquet-Léridon, C.; Jacolot, P.; Petit, N.; Marier, D.; Gadonna-Widehem, P. Effects of Maillard Reaction products on sensory and nutritional qualities of the traditional French baguette. J. Food Sci. 2018, 83, 2424–2431.
63. Zhang, L.; Xu, Y.; Li, Y.; Bao, T.; Gowd, V.; Chen, W. Protective property of mulberry digest against oxidative stress–A potential approach to ameliorate dietary acrylamide-induced cytotoxicity. Food Chem. 2017, 230, 306–315.
64. Nematollahi, A.; Kamankesh, M.; Hosseini, H.; Ghasemi, J.; Hosseini-Esfahani, F.; Mohammadi, A. Investigation and determination of acrylamide in the main group of cereal products using advanced microextraction method coupled with gas chromatography-mass spectrometry. J. Cereal Sci. 2019, 87, 157–164.

65. Svensson, K.; Abramsson, L.; Becker, W.; Glynn, A.; Hellenäs, K.E.; Lind, Y.; Rosen, J. Dietary intake of acrylamide in Sweden. Food Chem. Toxicol. 2003, 41, 1581–1586.

66. Şenyuva, H.Z.; Gökmen, V. Study of acrylamide in coffee using an improved liquid chromatography mass spectrometry method: Investigation of colour changes and acrylamide formation in coffee during roasting. Food Addit. Contam. 2005, 22, 214–220.

67. EU European Commission Recommendation of 3 May 2007 on the Monitoring of Acrylamide Levels in Food (2007/331/EC), L 123/33, 12.5.2007. 2007. Available online: (accessed on 18 March 2021).

68. Arámbula-Villa, G.; Flores-Casamayor, V.; Velés-Medina, J.J.; Salazar, R. Mitigating effect of calcium and magnesium on acrylamide formation in tortilla chips. Cereal Chem. 2018, 95, 94–97.

69. Lindsay, R.C.; Jang, S. Model systems for evaluating factors affecting acrylamide formation in deep fried foods. In Chemistry and Safety of Acrylamide in Food; Springer: Boston, MA, USA, 2005; pp. 329–341.

70. Saraji, M.; Javadian, S. Single-drop microextraction combined with gas chromatography-electron capture detection for the determination of acrylamide in food samples. Food Chem. 2019, 274, 55–60.

71. Norouzi, E.; Kamankesh, M.; Mohammadi, A.; Attaran, A. Acrylamide in bread samples: Determining using ultrasonic-assisted extraction and microextraction method followed by gas chromatography-mass spectrometry. J. Cereal Sci. 2018, 79, 1–5.

72. Alyousef, H.A.; Wang, H.; Al-Hajj, N.Q.; Koko, M. Determination of acrylamide levels in selected commercial and traditional foods in Syria. Trop. J. Pharm. Res. 2016, 15, 1275–1281.

73. Raffan, S.; Halford, N.G. Acrylamide in food: Progress in and prospects for genetic and agronomic solutions. Ann. Appl. Biol. 2019, 175, 259–281.

74. Eriksson, M.; Giovannini, S.; Ghosh, R.K. Is there a need for greater integration and shift in policy to tackle food waste? Insights from a review of European Union legislations. SN Appl. Sci. 2020, 2, 1347.

75. Commission Decision (2007/333/EC) Laying Down the Methods of Sampling and Analysis for the Official Control of the Levels of Lead, Cadmium, Mercury, Inorganic Tin, 3-MCPD and Benzo(A)-Pyrene in Foodstuffs. Available online: (accessed on 18 March 2021).

76. European Food Safety Authority. Update on acrylamide levels in food from monitoring years 2007 to 2010. EFSA J. 2012, 10, 2938.

77. European Food Safety Authority. Results on the monitoring of acrylamide levels in food. EFSA J. 2009, 7, RN-285.

78. FoodDrinkEurope (FDE). Acrylamide Toolbox. 2013. Available online: (accessed on 15 March 2021).

79. Wen, C.; Shi, X.; Wang, Z.; Gao, W.; Jiang, L.; Xiao, Q.; Liu, X.; Deng, F. Effects of metal ions on formation of acrylamide and 5-hydroxymethylfurfural in asparagine–glucose model system. Int. J. Food Sci. Technol. 2016, 51, 279–285.

80. EU European Commission Recommendation of 8 November 2013 on Investigations into the Levels of Acrylamide in Food (2013/647/EU). Available online: (accessed on 18 March 2021).

81. EU European Commission Regulation of 20 November 2017 Establishing Mitigation Measures and Benchmark Levels for the Reduction of the Presence of Acrylamide in Food (2017/2158). Available online: (accessed on 12 January 2020).

82. EFSA. Scientific opinion on the risks for public health related to the presence of furan and methylfurans in food. EFSA J. 2017, 15, 5005.

83. EU European Commission Recommendation of 7 November 2019 on the Monitoring of the Presence of Acrylamide in Certain Foods (2019/1888/EU). Commission Recommendation. Available online: (accessed on 18 March 2021).

84. EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain). Opinion of the Scientific Panel on Contaminants in Food Chain. EFSA J. 2005, 225, 1–27.

85. FDE. Food Drink Europe Acrylamide Toolbox 2019. Available online: (accessed on 15 March 2021).

86. Nguyen, H.T.; Van Boekel, M.A.J.S. Acrylamide and 5-hydroxymethylfurfural formation during biscuit baking. Part II: Effect of the ratio of reducing sugars and asparagine. Food Chem. 2017, 230, 14–23.

87. Suman, M.; Generotti, S.; Cirlini, M.; Dall'Asta, C. Acrylamide Reduction Strategy in Combination with Deoxynivalenol Mitigation in Industrial Biscuits Production. Toxins 2019, 11, 499.

88. Codex Alimentarius. Code of Practice for the Reduction of Acrylamide in Foods. CAC/RCP. 2009. Available online: (accessed on 20 December 2019).

89. Mildner-Szkudlarz, S.; Różańska, M.; Piechowska, P.; Waśkiewicz, A.; Zawirska-Wojtasiak, R. Effects of polyphenols on volatile profile and acrylamide formation in a model wheat bread system. Food Chem. 2019, 297, 125008.

90. Khorshidian, N.; Yousefi, M.; Shadnoush, M.; Siadat, S.D.; Mohammadi, M.; Mortazavian, A.M. Using probiotics for miti gation of acrylamide in food products: A mini review. Curr. Opin. Food Sci. 2020, 32, 67–75.