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# Outcomes of self-control plans on acrylamide levels in processed food

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### ABSTRACT

In 2002, researchers from Stockholm University discovered the presence of acrylamide (AA) in processed foods. This substance has been classified as "probably carcinogenic to humans" by the International Agency for Research on Cancer. In response to the alarming finding, the European Commission issued recommendations (2004/394/EC, 2010/307/EU, and 2013/647/EU), guiding food business operators, raising awareness, and promoting good manufacturing practices to minimize AA formation. These efforts laid the foundation for the comprehensive measures in Regulation (EU) 2017/2158. The Regulation implemented specific measures during production to reduce the amount of AA in food. This study monitored the AA levels in 15,674 samples from 12 processed food commodities. Potato-based products and coffee were found to be the main sources of AA exposure. The "baby foods" and "soft bread" food categories had the lowest contamination levels. The data were then compared to the information previously published by the European Food Safety Authority to assess the trend over time and the effectiveness of the mitigation measures. The results showed a decrease in AA contamination levels for most food categories, particularly for baby foods.

### 1. Introduction

Acrylamide (CAS No 79-06-01) is a water-soluble, low-molecularweight organic compound that has been used since the 1950s in various industrial applications, such as the production of polyacrylamides for wastewater and waste treatment and to manufacture paper, dyes, plastics, and many other household items. However, acrylamide (AA) can be toxic to biological organisms causing DNA damage, as well as neurological and reproductive effects (EFSA European food safety authority. 2015). In 1994, the International Agency for Research on Cancer (IARC, 1994) classified AA as 'probably carcinogenic to humans' (Group 2A). The general population may be exposed to AA through drinking water and tobacco smoke. In 2002, researchers from Stockholm University and the National Food Administration first detected the presence of AA in various cooked foods (Rosèn & Hellenäs, 2002). This finding highlighted how the diet can be an additional source of exposure to AA. AA is a by-product of the Maillard reaction that occurs between asparagine and reducing sugars when food is exposed to high temperatures, such as baking, frying, and roasting (Stadler et al., 2002). In light of the concerning discovery, the European Commission issued recommendation 2004/394/EC (Commission Recommendation, 2004), 2010/307/EU (Commission Recommendation, 2010), and 2013/647/EU (Commission Recommendation, 2013) with the aim of promoting safe manufacturing practices to reduce the formation of AA. According to EEC Regulation 315/93 (Council Regulation, 1993), AA is considered a process contaminant, which means that it is unintentionally present in foodstuffs as a residue from processing, including domestic cooking practices and industrial food production. The discovery of AA in food has led to numerous studies and monitoring that have confirmed its presence in many common foods. A recent review employed meta-analysis to compare the levels of AA among various food commodities, countries, and analytical techniques (Mousavi Khaneghah, Fakhri, Nematollahi, Seilani, & Vasseghian, 2022). AA has become a major issue in food safety management, requiring action from food producers. Furthermore, authorities and international bodies have made substantial efforts to assess the risks associated with AA and to understand the factors involved in its formation, in order to reduce its presence in the final food products. This activity also prompted food producers to adopt voluntary process control measures to minimize AA formation. "FoodDrinkEurope", a trade association representing the European food and beverage industry, has

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developed a 'toolbox' that collects information on how AA is formed in various foods and provides mitigation strategies (FDE, 2005-2019). In 2015, EFSA issued a scientific opinion on the presence of acrylamide in food, expressing concern about its carcinogenic effects and recommending further studies on the risks associated with its dietary intake (EFSA European food safety authority, 2015). According to the opinion, potato-based products, cereal based products, and coffee are the main contributors to the AA exposure. In 2017, the European Commission approved Regulation (EU) 2017/2158 (Commission Regulation, 2017) which established specific obligations for food business operators, including mitigation measures and benchmark levels for reducing the presence of AA in food. Since the production of AA depends on the composition of the food and the type of process it undergoes, Annex IV of the Regulation indicates different benchmark levels for various types of food products such as potato products, bakery products, cereal products, coffee, and baby foods, etc. These benchmark levels are not considered maximum limits of non-compliance, but rather useful indicators for verifying the effectiveness of mitigation measures implemented by food business operators to achieve levels of AA "as low as reasonably achievable".

Moreover, to ensure harmonized application and enforcement across the EU, the Standing Committee on Plants, Animals, Food and Feed (PAFF Committee) approved the 'Guidelines on the Implementation of Commission Regulation (EU) 2017/2158' (PAFF, 2018). In 2019, the European Commission adopted Recommendation (EU) 2019/1888 (Commission Recommendation, 2019) which acknowledged the insufficient data on the presence of AA in foods not falling within the scope of the Regulation (EU) 2017/2158 (Commission Regulation, 2017), as well as certain foods referred to in Article 1(2) of this Regulation. In the same year, the Commission Implementing Regulation (EU) 2019/2093 updated the performance criteria for methods of analysis for certain contaminants in food, including AA. Currently, the European Parliament is examining the proposal to amend Regulation (EC) No 1881/2006 (Commission Regulation, 2006) concerning the maximum levels of AA in certain foodstuffs for infants and young children. The aim of this study was to evaluate the outcomes of a two-year analysis of AA levels in processed foods using a single accredited analytical approach applied to a large variety of samples. The findings were then compared with the EFSA's published data to assess the trend over time and the effectiveness of the mitigation measures specified in Regulation (EU) 2017/2158 (Commission Regulation, 2017) and implemented by food producers.

### 2. Material and methods

### 2.1. Sample collection

The reported data pertain to a total of 15,674 food samples collected from 390 different food producers between 2020 and 2022 and analyzed before commercialization.

The number of samples and the food categories examined, as listed according to the EFSA Scientific Opinion on acrylamide in food (EFSA European food safety authority, 2015), are reported in Tables 1 and 2.

## 2.2. Sample preparation and analysis

The method used in this study was developed in accordance with the European Standard EN 16618:2015 "Food analysis - Determination of acrylamide in food by liquid chromatography tandem mass spectrometry (LC-ESI-MS/MS)" (CEN Comité Européen de Normalisation, 2015). The method was applied as such, specifically validated to cover all the matrices included in the scope of the analysis and accredited according to the ISO-17025:2018 Standard (accreditation n. 0051 by Accredia). The solvents used were of HPLC grade for the extraction procedure, or LC-MS grade for HPLC-MS/MS analysis. AA standards were purchased from Sigma-Aldrich or Supelco, Sigma-Aldrich (Saint Louis, MO, USA), 2,3,3-d3-acrylamide was obtained from HPC Standards GmbH (Cunnersdorf, Germany).

In brief, the sample, spiked with the labeled internal standard, was extracted with solvent and then defatted with n-hexane. The sample extract was then cleaned-up using SPE resins, concentrated, and filtered before LC-MS/MS analysis. Samples were generally analyzed in batches of ten to fifty, which included a process blank, a reagent blank, a spiked sample, and reference materials as calibration curve. The method and process were monitored by periodically carrying out quality controls on method performance, such as repeatability and trueness, through participation in interlaboratory proficiency tests.

The analytical system consisted of an Agilent 1290 LC System binary pump equipped with autosampler, degasser and column heater fitted with a Hypercarb graphite column (ThermoFisher Scientific, Waltham, MA, USA) coupled to an Agilent 6460 QQQ DSP version triple quadrupole mass spectrometer equipped with a jet stream source operating in the positive electrospray ionization (ESI+) mode. The instrument was operated in the multiple reaction monitoring mode (MRM), and the following transitions were monitored: AA (72 > 55 and 72 > 44); 2,3,3-d<sub>3</sub>-AA (75 > 58). Data acquisition and processing were performed using Agilent MassHunter quantitative analysis software.

Table 1
Overview of the result of analyses on samples of the various food categories and number and percentage of samples exceeding the benchmark levels (BL) set by Regulation (EU) 2017/2158.

Food category	Total samples	Left-censored data				Quantifiable samples		Reg. EU 2017/2158 Benchmark Level (BL)		
	number	<lod< th=""><th></th><th>LOD &lt; x</th><th>&lt; LOQ</th><th>x &gt; LOQ</th><th><u> </u></th><th>BL</th><th>x &gt; BL</th><th></th></lod<>		LOD < x	< LOQ	x > LOQ	<u> </u>	BL	x > BL	
		number	%	number	%	number	%	μg/kg	n°	%
Potato fried products (except potato crisps and snacks)	536	117	21.8%	47	8.8%	372	69.4%	500	56	10.4%
Potato crisps and snacks	5858	13	0.2%	39	0.7%	5806	99.1%	750	1597	27.3%
Soft bread	3099	330	10.6%	955	30.8%	1814	58.5%	50	269	8.7%
Breakfast cereals	532	28	5.3%	113	21.2%	391	73.5%	300	49	9.2%
Biscuits and wafer	2496	17	0.7%	107	4.3%	2372	95.0%	350	282	11.3%
Crackers	237	1	0.4%	4	1.7%	232	97.9%	400	24	10.1%
Crisp bread	452	1	0.2%	11	2.4%	440	97.3%	350	42	9.3%
Roasted coffee (dry)	1285	0	0.0%	9	0.7%	1276	99.3%	400	40	3.1%
Instant coffee (dry)	212	1	0.5%	20	9.4%	191	90.1%	850	6	2.8%
Coffee substitutes (dry) based on cereals	26	2	7.7%	1	3.8%	23	88.5%	500	0	0%
Baby foods. other than cereal-based	592	301	50.8%	135	22.8%	156	26.4%	40	11	1.9%
Processed cereal-based baby foods	349	177	50.7%	75	21.5%	97	27.8%	40	75	21.5%

Comparison of the data measured in this study and those reported in the 2015 EFSA Scientific Opinion on acrylamide in food between the two sources (European Countries-EU and Food Associations-FA). Mean, median

and P95 values (μg/Kg) are presented as the middle-bound estimate.

Food category	Present study	t study				EFSA E	uropean foo	od safety au	EFSA European food safety authority, 2015	15							
						Europe	European Countries (EC)	s (EC)			Food Asso	Food Associations (FA)	(A <sup>7</sup>			EC + FA	
	п	%DT	Mean	Median	P95	п	%OT	Mean	Median	P95	п	%DT	Mean	Median	P95	Mean	P95
Potato fried products (except potato crisps and snacks)	536	30.6%	213	100	757	1378	13.9%	332	196	1115	316	15.8%	201	170	493	308	971
Potato crisps and snacks	5858	%6.0	649	527	1630	800	7.0%	280	389	1841	33,701	%0	384	310	920	389	932
Soft bread	3099	41.5%	22	13	70	535	49.5%	40	17	137	∞	%0	181	180	\	42	156
Breakfast cereals	532	26.5%	66	44	428	561	29.1%	113	29	348	699	1.3%	201	128	661	161	552
Biscuits and wafer	2496	2.0%	171	112	528	682	21.1%	201	103	810	Data not provided	provided				\	\
Crackers	237	2.1%	177	80	602	162	12.3%	231	183	290	Data not provided	provided				\	\
Crisp bread	452	2.7%	145	99	553	437	25.2%	149	68	428	91	%0	277	278	520	171	486
Roasted coffee (dry)	1285	0.7%	236	226	384	999	7.2%	244	203	563	56	%0	363	360	\	249	543
Instant coffee (dry)	212	%6.6	455	464	735	116	9.8	674	620	1133	746	%0	716	029	1115	710	1122
Coffee substitutes (dry) based on cereals	56	11.5%	128	121	\	88	%8.9	1499	299	4500	Data not 1	provided				_	\
Baby foods. other than cereal-based	592	73.6%	8	2	28	348	70.1%	24	15	20	89	20.0%	24	8	123	24	72
Processed cereal-based baby foods	349	72.2%	39	3	193	394	48.2%	103	15	200	342	4.7%	38	17	154	73	175

# number of samples; LC%, left-censored samples percentage; P95, 95th percentile contamination level.

### 2.3. Data management

A descriptive statistical approach was used to analyze the levels of AA detected in each food category. The concentrations of AA measured in this study were compared to the data reported in the EFSA Opinion (EFSA European food safety authority, 2015). The middle bound (MB) approach was used for making a comparison while considering left-censored data: all samples below the limit of detection (LOD) and limit of quantification (LOQ) values were assumed to be half of the respective LOD and LOQ values (EFSA European food safety authority, 2010). Therefore, the mean, median and 95th percentile contamination levels were calculated as the middle bound (MB) estimate. According to EFSA (EFSA European food safety authority, 2015), in case of too few observations (less than 60 for the 95th percentile), the estimation may be biased, and thus, was not provided.

### 3. Result and discussion

### 3.1. Method quality assurance

As mentioned in Section 2.2, the method for determining AA in food is accredited according to ISO-17025:2018 standards. To assess the method's specific performances across different food categories, fortified samples were subjected to repeated measurements at three distinct concentration levels, including the limit of quantification (LOQ). The resulting data are summarized in Table 3.

The labeled internal standard was used to compensate for matrix effects, imprecision, and recovery issues. The linearity of the method was proved by the correlation coefficient ( $R^2$ ) always being  $\geq 0.98$  within the concentration ranges shown in Table 3. To estimate measurement uncertainty, the uncertainty components associated with systematic effects on mass and volume measurements (as well as method precision, recovery, and calibration curve) were assessed. Since no type-A estimates were obtained from less than ten repeated measurements, k=2 was chosen as a recovery factor. Details of the resulting relative expanded uncertainty for each food category are provided in Table 3.

# 3.2. Acrylamide levels measured in food matrices

It is important to note that the significant amount of data included in this work was not obtained from different monitoring sources. Instead, it was obtained using the same analytical method. Moreover, this method meets all the performance criteria required for accreditation. This aligns with EFSA's Scientific Opinion on AA in food (EFSA European food safety authority, 2015) which recommends that any future monitoring program for AA in foods should employ consistent analytical techniques and collect enough samples from each food category to produce statistically reliable results.

Table 1 displays the corresponding limits of detection (LOD) and limits of quantification (LOQ). It provides an overview of the results, broken down by the number and percentage of samples with non-detectable concentrations (<LOD), non-quantifiable samples (LOD < x < LOQ), and quantifiable samples (>LOQ), all expressed in  $\mu g/kg$ . The last three columns show the benchmark levels set by Regulation (EU) 2017/2158 (Commission Regulation, 2017), as well as the number and percentage of samples that exceeded these concentration levels.

Table 2 compares, for each food category, the total number of samples, the total percentage of left-censored results, the mean, median and 95th percentile contamination level of the measured concentrations of AA, as the middle-bound estimate. The corresponding values from the EFSA Opinion are provided for both European Countries (EC) and Food Associations (FA), as well as a combined total for both (EC + FA). It is essential to consider appropriate strategies for handling non-quantified (<LOQ) and non-detected (<LOD) results when conducting food contamination monitoring. These samples are assumed to have concentrations lower than these limits. The numerical value used to replace

**Table 3**Performance characteristics of the method in relation to the various food categories.

Food category	LOD	LOQ	Recovery	Precision	Linearity range	Uncertainty
	μg/kg	μg/kg	%	CV%	μg/kg	%
Potato fried products (except potato crisps and snacks)	6	20	98.6	2.2	20-2500	12
Potato crisps and snacks	6	20	97.1	1.6	20-2500	12
Soft bread	3	10	97.6	1.4	10-1250	11
Breakfast cereals	6	20	97.1	1.6	20-2500	12
Biscuits and wafer	3	10	97.6	1.4	10-1250	11
Crackers	3	10	97.6	1.4	10-1250	11
Crisp bread	3	10	97.6	1.4	10-1250	11
Roasted coffee (dry)	15	50	99.7	1.1	50-5000	14
Instant coffee (dry)	15	50	99.7	1.1	50-5000	14
Coffee substitutes (dry). based on cereals	15	50	97.1	1.6	50-5000	12
Baby foods. other than cereal-based	3	10	97.0 <sup>a</sup>	2.0 <sup>a</sup>	10-1250	11
			98.6ª	2.2 <sup>a</sup>		
			99.1 <sup>a</sup>	3.0 <sup>a</sup>		
Processed cereal-based baby foods	6	20	97.1	1.6	20-2500	11

LOD, limit of detection; LOO, limit of quantification; CV, coefficient of variation.

these results can significantly influence the estimation of dietary exposure, depending on the amount of non-quantified and non-detected results in the data related to the concentration of the analyte in various food categories. To simplify the comparison between the data obtained in this study and those reported in the EFSA Scientific Opinion on AA in food (EFSA European food safety authority, 2015), the middle-bound approach was used.

The highest levels of AA were found in "Potato crisps and snacks" with the highest MB values, with a mean of 649  $\mu$ g/kg, a median of 527  $\mu$ g/kg, and a 95th percentile of 1630  $\mu$ g/kg "Instant coffee (dry)" had the second-highest levels, with a mean of 455  $\mu$ g/kg, a median of 464  $\mu$ g/kg, and a 95th percentile of 735  $\mu$ g/kg.

The food categories with the lowest contamination levels were as follows: "Baby foods, other than cereal-based" (mean 8  $\mu$ g/kg, median 2  $\mu$ g/kg, and 95th percentile 28  $\mu$ g/kg), "Soft bread" (mean 22  $\mu$ g/kg, median 13  $\mu$ g/kg, and 95th percentile 70  $\mu$ g/kg), and "Processed cereal-based baby foods" (mean 39  $\mu$ g/kg, median 3  $\mu$ g/kg, and 95th percentile 193  $\mu$ g/kg). When examining the proportion of samples from each food category that exceeded the benchmark level set by Regulation (EU) 2017/2158 (Commission Regulation, 2017), it was found that "Potato crisps and snacks" had the highest rate at 27.3%, followed by "Processed cereal-based baby foods" at 21.5%, "Biscuits and wafers" at 11.3%, "Potato fried products (except potato crisps and snacks)" at 10.4%, and "Crackers" at 10.1%.

## 3.3. Comparison with EFSA datasets

A total of 15,674 food samples were analyzed for AA levels before being placed on the market. These results were compared to those reported by EFSA (EFSA European food safety authority, 2015) in 42,037 samples from the same food categories. The data were collected by EFSA from two distinct sources: European countries and food associations. The data from European countries were mostly generated within the framework of official monitoring programs (6067 samples). The food association samples (35,970 samples) were mostly taken at the manufacturing or storage place by six organizations: four European food associations (European Coffee Federation, European Breakfast Cereals Association, European Snacks Association, FoodDrinkEurope) and two national associations (Finnish Food and Drink Industries' Federation and the German Plant Bakeries Association). It should be noted that 94% of these samples (33,701) belong to the food category "Potato crisps and snacks".

Table 2 compares the levels of AA measured in this study to those reported by EFSA (EFSA European food safety authority, 2015) for both the European countries (EC) and the food association (FA) sources. For most food categories, the mean values in this study are lower and are

more consistent with those obtained by the EC than those reported by the FA. However, the data related to the mean contamination measured for "Processed cereal-based baby foods" is an exception, as it is more than double the EC values but almost the same as those reported by the FA. Additionally, the AA contamination in "Baby foods other than cereal-based" is 67% less than both the EC and FA data. The only other food category with mean values higher than the EC (+12%) and FA (+69%) is "Potato crisps and snacks".

The median levels of AA measured in this study are generally lower than those reported in the EFSA Opinion, except for the "Potato crisps and snacks" category which has notably higher values: 35% higher than the EC and 70% higher than the FA. The specific comparison with EC data for "Roasted coffee (dry)" and "Biscuits and wafers" shows an increase of 11% and 9% respectively. However, the median value for "Processed cereal-based baby foods" is significantly lower than both the EC value (-80%) and the FA value (-82%). This contrasts with the comparison of mean values, indicating that the means are biased by outliers

The 95th percentile values can provide a good indication of the upper limit of contamination levels and can be useful in setting health or safety standards. A comparison of the datasets reveals that the measured contamination values are lower than those reported by the EC, but higher than the limited data available from FA. When compared to both the EC and FA datasets, "Crisp bread" has the highest values, with increases of 29% and 6% respectively. The values for "Breakfast cereals" and "Crackers" are slightly higher than those recorded by the EC, with increases of 23% and 2% respectively. The data provided by the FA shows that the variations for certain food categories are much greater than for others: "Potato crisps and snacks" +77%, "Potato fried products (except potato crisps and snack)" +54%, and "Processed cereal-based baby foods" +25%.

Regulation (EU) 2017/2158 (Commission Regulation, 2017) includes mitigation measures for each food category based on current scientific and technical knowledge. These measures generally pertain to the selection and agronomy of raw materials, storage and transport, product design, processing, and heating. The latest peer-reviewed study provides compelling evidence to demonstrate that the legislation is successfully working and that it is feasible to achieve a reduction in AA over time without compromising the sensory values of the final product.

The effectiveness of the mitigation measures has been proven in potato crisps since the release of the Regulation, with a decrease of 55.3% compared to 2004 and a 10.3% reduction compared to 2008 (Mesias et al., 2020). The values, referring to 70 samples of potato chips marketed in Spain (mean: 664  $\mu g/kg$ , median: 569, 95th percentile: 1576), are consistent with those obtained in the present work (see Table 2). Coffee is another significant source of AA in the food category.

<sup>&</sup>lt;sup>a</sup> depending on the principal ingredient: the options are fruit/vegetables, meat/fish, and milk derivatives, respectively.

In a review conducted by Schouten et al., 2020, various mitigation strategies for AA formation in coffee throughout different production stages were examined. The study concluded that the most effective methods for achieving low levels of AA in the final coffee product include selecting the highest quality Arabica green coffee variety, applying high roasting thermal input, and employing shorter brewing techniques. These findings align with the mitigation measures outlined in Regulation (EU) 2017/2158 (Commission Regulation, 2017). Furthermore, the study proposed innovative interventions for AA control in coffee, such as enzymatic treatments of raw materials, vacuum or steam roasting, supercritical fluid extraction of roasted beans, and final beverage treatments involving yeast fermentation and the addition of amino acids or additives. Recently, the levels of AA in baby foods have been reviewed, along with the progress made in current regulations to reduce its presence (Boyaci-Gunduz, 2022).

A review by Bachir et al., 2022, provides a comprehensive summary of both innovative and conventional techniques for mitigating AA in foods. The review covers various approaches, including the use of lactic acid bacteria, yeasts, and cell extracts, as well as conventional methods like blanching and microwave heating. Recent advancements in pretreatments, post-baking processing, and alternative baking technologies to mitigate AA formation have been reviewed (Suparna Devu et al., 2022). The search for new approaches to complement the existing ones is constantly evolving. However, it is essential for these strategies to be technically applicable at the industrial level while maintaining the sensory characteristics of foods.

### 4. Conclusion

Several processed food products may contain toxic molecules that are considered risk factors for human health. Monitoring AA levels in foodstuffs can provide useful data for proper risk assessment for consumers and enable the verification of innovative measures and strategies on food preparation processes implemented to reduce this contamination.

Our results are consistent with the data reported in the EFSA European food safety authority, 2015; however, lower levels of contamination were recorded for most food categories. Processed potato products were found to be the most contaminated, followed by coffee. These data should be further positively evaluated considering that the samples analyzed were part of the self-control plan. Therefore, the introduction on the market of those foods that exceeded the benchmark level set by Regulation (EU) 2017/2158 (Commission Regulation, 2017) has been avoided. Of particular note is the median contamination figure recorded for baby foods. These showed the lowest levels among the food categories and a five-fold decrease compared to those recorded in the EFSA Opinion. This monitoring confirms the widespread presence of AA in a wide range of food commodities, but also demonstrates the usefulness of the control and mitigation measures prescribed by European legislation and implemented by food manufacturers in the processing of their products and emphasizes the need for continued monitoring and enforcement.

# CRediT authorship contribution statement

Giulia Rampazzo: Conceptualization, Data curation, Writing – original draft, Visualization. Michela Casarotto: Formal analysis, Investigation, Validation, Writing – review & editing. Claudia Finotello: Formal analysis, Investigation, Validation, Writing – review & editing. Marco Redaelli: Resources, Validation, Writing – review & editing. Giampiero Pagliuca: Conceptualization, Data curation, Writing – review & editing, Supervision. Teresa Gazzotti: Data curation, Writing – original draft, Visualization, Supervision.

### **Declaration of competing interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Data availability

The data that has been used is confidential.

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