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Results of the BfR MEAL Study: Acrylamide in foods from the German market with highest levels in vegetable crisps

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A B S T R A C T

Acrylamide (AA) is formed in foods due to thermal processes. AA was analysed in 230 foods in the first German Total Diet Study and the highest mean levels of AA were found in vegetable crisps (1430 µg/kg), followed by potato pancakes (558) µg/kg) and pan-fried potatoes (450 µg/kg). In various foods, e.g. French fries and sweet potatoes, AA was also tested for different browning degrees and cooking methods. French fries cooked to a browning degree of 3 in all cooking methods exceeded the benchmark level set by the European Union. French fries prepared in the oven and sweet potatoes in the air fryer had the lowest AA levels. In foods from the German market, AA was found also in foods such as popcorn (243 µg/kg), salty sticks (190 µg/kg), and dark chocolate (130 µg/kg). Levels of AA found in our study may support future dietary exposure and food safety assessments.

1. Introduction

Thermal processing is an important treatment step during the production of various foods, either on an industrial scale or in the domestic kitchen, which has long been used for food preservation or to improve salubriousness and sensory properties of foods. On the other hand, food is a complex matrix of nutritive substances (e.g., proteins/amino acids, carbohydrates, fats/fatty acids or vitamins and minerals) and other natural ingredients that can be chemically converted or react with each other during thermal treatment. This can lead to desirable changes in colour, flavor or texture, but also to the formation of undesirable heat-induced toxic compounds, commonly referred to as thermal process contaminants in foods. A number of substances such as acrylamide (AA) have been identified in foods in recent years and require particular attention due to potential health concerns. The discovery of AA as a chemical hazard in food was first reported in April 2002 by the Swedish National Food Authority (SNFA) in collaboration with researchers from Stockholm University (Tareke, Rydberg, Karlsson, Eriksson, & Törnqvist, 2002). Frying, roasting or baking results in a multitude of Maillard reaction products giving these foods a typical taste and odor. Among these, however, AA is formed as an undesired contaminant when foods containing the amino acid asparagine and certain reducing sugars are prepared at temperatures typically higher than 120 °C under low moisture conditions (Mottram, Wedzicha, & Dodson, 2002). Since the first discovery of AA in food, numerous factors have been recognized that may affect the AA formation in foods, including ingredient composition and product formulations (e.g., levels of carbohydrates, free asparagine, reducing sugars, pH, water activity, use of ammonium bicarbonate, concentrations of competing amino acids). Consequently, AA has been found in higher amounts in fried potato products (potato chips and French fries), in cereal-grain-based baked goods (cookies, crackers, breakfast cereals, toasted bread), or roasted coffee (EFSA, 2015b). The predominant toxic effect of the toxicological profile of AA is the genotoxicity (in vitro and in vivo) and the carcinogenicity observed in animal studies with the potential to cause cancer in humans (EFSA, 2015b; EFSA, 2022). AA has been classified by the International Agency for Research on Cancer (IARC) as a Group 2 A carcinogen (probably carcinogenic to humans) (IARC, 1994). AA itself is an α,β-unsaturated carbonyl compound with electrophilic reactivity, which reacts with nucleophilic groups in biological molecules and thus may contribute to the generation of weak genotoxic potential and other toxic effects. However, the genotoxic effect of AA observed in animal studies is primarily attributed to the formation of DNA adducts promoted by the reactive epoxide glycidamide (GA) as a metabolite of AA (EFSA, 2015b;

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EFSA, 2022). In addition to animal studies, a large number of epidemiological studies have been published examining the evidence for an association between dietary exposure to AA and cancer, but overall the epidemiological evidence is still inconsistent (EFSA, 2022). Considering the increasing knowledge on the factors that might affect the extent of AA formation in food, risk management options have been established in the EU. They are based on (i) a collection of mitigation measures, sampling and analysis requirements for food monitoring (EU VO 9152023) and (iii) benchmark levels as performance indicators to be used to verify the effectiveness of the mitigation measures which are based on experience and occurrence for broad food categories such as potato products (e.g. French fries and chips), bread, breakfast cereals, biscuits and wafers (e.g. crackers, crispbread and gingerbread), coffee (roasted coffee, instant coffee and coffee substitutes) and infant formula (EC, 2017). The BfR MEAL Study (meals for exposure assessment and analysis of food) is the first German Total Diet Study (TDS) and started the field phase end of 2016 (Sarvan, Burgelt, Lindtner, & Greiner, 2017). A TDS is a cost-effective and reliable method to analyse substances in foods for exposure assessment (EFSA/FAO/WHO, 2011) and has been performed in many countries. The design of a TDS includes three criteria: i) it must be representative of the dietary behaviour of a population, ii) foods must be prepared as they are eaten, and iii) similar foods are grouped together. For food preparation, domestic cooking techniques are used to depict possible influence on food composition, such as degradation or formation of compounds (EFSA/FAO/WHO, 2011). A TDS provides a comprehensive database of average levels of substances in foods, which can be used for chronic dietary exposure assessment (EFSA/FAO/WHO, 2011). The objective of this study is to provide a current and comprehensive data set on average levels of AA in foods as typically prepared and consumed in households in Germany. Furthermore, the impact of different cooking methods and of browning degrees in foods was investigated. This research will provide a comprehensive database of average levels of AA in foods, which can be subsequently used for chronic dietary exposure assessments, risk management and risk communication.

2. Material and methods

2.1. Sample collection and preparation

The BfR MEAL study follows the design of a TDS. The specific design is described in detail elsewhere (Sarvan et al., 2017). Based on the data set of the National Consumption Survey II (NVS II) (Heuer, Krems, Moon, Brombach, & Hoffmann, 2015) covering age groups 14 to <80 years and a German consumption data set for children called VELS ("Consumption Survey of Food Intake among Infants and Young Children") (Banasiak, Heseker, Sieke, Sommerfeld, & Vohmann, 2005) covering age groups 0.5 to <5 years, a MEAL food list was created (Sarvan et al., 2017). The most consumed foods were selected from the respective main food group, using FoodEx2 classification (EFSA, 2015a). The MEAL food list included 365 different foods in total, covering at least 90% of the average German diet for different age groups. Foods known to contain high levels of relevant substances were included as well, even if consumed rarely (<10% of consumption). In order to represent German purchasing behaviour (e.g., brand, type, production method, type of packaging, type of storage conditions, and/or origin), market data from a representative panel of 30,000 households were purchased and analysed over a 12-month period (between 2015 and 2017) (Sarvan et al., 2017). After delivery of the sampled foods to the BfR MEAL kitchen, they were labelled, photographed and stored appropriately until preparation. All samples were prepared as usually consumed. To replicate German household behaviour, an online survey on browning degree (BD) preferences ($n = 2000$) as well as a telephone based survey ($n = 1000$) on the preferred material of used kitchen utensils were conducted (Hackethal et al., 2023). Only BDs and processing methods with >5% of preference were investigated for AA levels. Ingredients with a proportion of <5% (*w*/w) of the recipe as well as tap water were not selected representatively. Instead, standard ingredients were used by default (e.g. tap water from the kitchen for cooking or standardized oil, salt and spices). Following preparation, similar foods were pooled into composite samples according to their relative consumption habits and homogenized using a Grindomix GM300 blender (Retsch GmbH, Haan, Germany). Liquid nitrogen was added for samples to be analysed on AA to avoid degradation during homogenization. The homogenized material was stored in 100 mL polypropylene (PP) vessels for samples to be analysed for AA. All samples were stored at −20 °C until analysis. For each substance investigated within the study, the MEAL food list was adjusted, to sample and analyse only relevant foods. In total, 393 pooled samples of 230 foods were analysed for AA, consisting of 5 to 20 individual food items (subsamples). The amount of individual food items depended on the reason of the selection of the pooled food. The reasons for selecting foods for AA analysis were: i) foods on the food list that have been heated were examined (20 subsamples), to create a sound data basis for background exposure to AA via food. ii) As the effect of preparation in households on AA is less investigated than in industrial processes, frequently used methods used in private households (frying, roasting, grilling, baking) were analysed separately. If more than one method was investigated, the number of subsamples was reduced to 5 subsamples. The temperatures, cooking times and processing techniques were adapted for each pool. For example, the pools "sweet potato fries" and "French fries" were prepared in the oven, in the air fryer and in the deep fryer. The pool "popcorn" was prepared in the microwave, in the oven and in the griller. iii) Due to the impact of temperature and time on AA levels during heat treatment, different browning degrees were investigated in 143 foods typically prepared in the household and known to generate substantial levels of AA. The BDs were prepared with increasing cooking times and categorized from 1 to 5 (lowest to highest). For instance, the pool "sweet potato fries" was cooked at 170 °C for 3 min for BD 1 and at 170 °C for 7 min for BD 2. Foods were only tested for the BDs in which they are commonly consumed in Germany, according to the consumer's surveys conducted for the MEAL study (Hackethal et al., 2023). The internal temperature of the samples tested for different browning degrees was measured with a puncture thermometer (Extech Instruments, Switzerland) at the end of the cooking process.

2.2. Acrylamide analysis

For the analysis of AA, an external accredited laboratory was contracted. The laboratory had to demonstrate several years of extensive experience in the determination of analytes in various food samples. AA was extracted from the homogenized samples using an acetonitrile/water mixture (Th. Geyer GmbH & CO. KG, DE; Sartorius AG, DE). Approximately 2.5 g of homogenized sample material was weighed into a 50 mL disposable centrifuge tube and 50 μL of internal standard (D3 acrylamide) (LGC Limited, GB) was added. After adding 12.5 mL of water and 12.5 mL of acetonitrile, the mixture was heated in a water bath at 60 °C to 70 °C for 15 min with occasional shaking. This was followed by extraction in an ultrasonic bath for 20 min (SONOREX, BANDELIN electronic GmbH Co. KG, DE). After cooling and centrifugation (Eppendorf SE, DE) at 3200*g* for 5 min, 1 mL of the mixture was applied to a self-packed solid phase cartridge with silica gel phase (Phenomenex Ltd., DE) and ion exchange solvent (Phenomenex Ltd., DE). After 15 min the analytes were eluted twice with 7 mL ethyl acetate (Th. Geyer GmbH & CO. KG, DE) with an interval of 15 min. The eluates were combined in a 40 mL EPA vial and subsequently brought almost to dryness, the residue was resuspended in 0.4 mL of water, centrifuged and transferred to an amber glass containing micro-insert. A dilution factor of 10 was applied. Acrylamide was quantified by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) (Agilent Technologies Deutschland GmbH, DE; AB Sciex API 4000, US) A run lasted 15 min and the eluent used was water (ultrapure) with 0.1%

acetic acid (VWR International GmbH, DE) at a flow rate of 0,3 mL/min. A column (Hypercarb 150 × 2.1 mm, 5 μm, ThermoFisher Scientific, US) with an attached pre-column (Hypercarb 10 × 2.1, 5 μm Drop-in Guard, ThermoFisher Scientific, US) was used for analysis. The intra-laboratory method validation was performed with recoveries between 88%–98% and intermediate precision between 5.9%–9.4% depending on the AA level. The validation data thus meet the performance criteria for the analysis of AA according to Commission Regulation (EC) No 333/2007. As an external quality control, proficiency testing was performed between 2016 and 2019 for different available matrices such as roasted coffee (LVU 34), coffee (FAPAS 3078), biscuits (FAPAS 3075, 3084), cocoa (BVL PZK0419) and baby biscuits (BVL PZK0419) with z-scores between −0.5 and 1.0. Appropriate reference materials (chocolate, cookies and coffee powder) were used in each sequence depending on the matrix to ensure data quality on a daily basis. The limit of detection (LOD) was 6 μg/kg and the limit of quantification (LOQ) was 20 μg/kg. The extended measurement uncertainty of the method (k = 2) was estimated to be 20%.

2.3. Statistical analysis

Results below LOD or LOQ were substituted using the upper bound (UB) approach, i.e. results below the LOD were substituted by the value reported as the LOD and results below the LOQ and above the LOD were substituted by the value reported as the LOQ. In addition, the modified lower bound (mLB) approach was calculated by replacing results below the LOD to zero and by replacing results below the LOQ and above the LOD by the value reported as the LOD. As AA is a substance of health concern, the conservative UB approach is given in the present study. The results of the UB and mLB approach in the main food groups are available in the Supplementary data (Table S1 and Table S2, respectively). For the calculation of the mean AA levels in foods, only the most consumed browning degrees and the most preferred preparation methods (when available) were included, according to the survey data published by Hackethal et al. (2023). For popcorn, all three preparation methods (griller, oven and stove) were included due to lack of information regarding the most used one in Germany. All mean levels for different browning degrees and preparation methods are included in the supplementary data (Table S3). Calculation of mean, median (50th percentile, P50), 95th percentile (P95), minimum (min) and maximum (max) values of the main food groups were carried out using Microsoft Excel 2016 (version 16.0.5400.1000) and are given in Table S2 in the Supplementary data. The P95 was calculated only for main food groups of at least 20 pooled samples using the QUANTIL.EXKL command.

3. Results and discussion

Acrylamide was quantified in 137 out of 393 (35%) pooled samples included in this study. The food groups that showed the highest mean AA levels were: “Starchy roots or tubers and products thereof, sugar plants” (328 μg/kg), “Composite dishes” (100 μg/kg) and “Vegetables and vegetables products” (81 μg/kg) (Fig. S1, Supplementary data). The group “Starchy roots or tubers and products, thereof, sugar plants” was composed only by potato-based foods, such as French fries, potato crisps and sweet potato fries, contributing to the high AA levels in this group. Similarly, in the group “Composite dishes”, foods prepared with potatoes such as omelette with potatoes and bacon (German: Bauernfrühstück) and potato pancakes (German: Kartoffelpuffer), contributed the most to the high AA levels. The group “Vegetables and vegetables products”, included mostly cooked vegetables, which contained low levels of AA. However, the pool vegetable crisps included in this group, had high AA levels. In 256 (65%) pooled samples, AA levels were below the LOQ or below the LOD. The food groups with the lowest mean AA levels were “milk and dairy products” and “eggs and egg products” (Table S1, Supplementary data). EFSA reported similar AA levels in the food categories “potato fried products” (308 μg/kg) and “potato crisps and snacks” (389 μg/kg) (EFSA, 2015b).

The 15 MEAL foods with the highest AA levels are shown in Fig. 1. In all foods, the highest mean levels were measured in vegetable crisps (1430 μg/kg), followed by potato pancakes (558 μg/kg) and by pan-fried potatoes (German: Bratkartoffeln) (450 μg/kg). Other potato-based foods such as potato crisps, sweet potato fries and oven-baked crisps also had high levels of AA (190 μg/kg, 180 μg/kg and140 μg/kg, respectively).

3.1. Vegetable crisps

Vegetable crisps have been widely available to consumers since some years. Instead of using potatoes as the main raw ingredient, vegetable crisps are prepared e.g. with beetroot, parsnip, sweet potato or carrot. In our study, vegetable crisps were the food with the highest levels of AA (1430 μg/kg), containing almost eight times more AA than potato crisps (190 μg/kg). EFSA reported similar AA levels in vegetable crisps (1846 μg/kg) (EFSA, 2015b). Other studies in Germany have also measured high levels of AA in crisps made from sweet potato, parsnip, carrot or beetroot (240–2100 μg/kg), while crisps made from cabbage, kale, courgette or radish showed lower levels of AA (<LOD (10 μg/kg) to 90 μg/kg) (Breitling-Utzmann & Hankele, 2019; Oellig, Gottstein, & Granvogl, 2022). The amount of AA developed in vegetable crisps seems to be positively correlated with the initial levels of reducing sugars in the fresh vegetables, which might explain the differences between vegetable crisps (Breitling-Utzmann & Hankele, 2019). Furthermore, the temperature and cooking time might be crucial for the development of AA in vegetable crisps. In order to reduce the AA uptake, Breitling-Utzmann and Hankele (2019) stated, that oven-baked crisps should be preferred over deep-fried varieties and that the oven temperature should be below 140 °C. In our study, the measured AA levels in vegetable crisps were twice as high as the benchmark level set by the European Commission (EC) for potato crisps (750 μg/kg). A benchmark level for vegetable crisps has not yet been set up by the EC; however, the EC recommends that vegetable crisps should be monitored for AA levels (EC, 2019).

3.2. Potato products

As expected, potato-based products showed high levels of AA compared to other foods in our study. The second and the third foods with the highest AA content were potato pancakes (558 μg/kg) and pan-fried potatoes (450 μg/kg), respectively. Potato pancakes, a mixture of eggs, flour and potatoes, are typically fried in a frying pan or in the deep fryer at temperatures around 170–175 °C, and therefore highly susceptible to the development of AA. Likewise, pan-fried potatoes are also exposed to high temperatures during preparation and some recipes recommend frying them for about 5–7 min, which can lead to high levels of AA. In our study, potato pancakes and pan-fried potatoes samples were prepared according to different BDs (Fig. 2). As reported in the MEAL survey, 53.5% and 33.9% of German consumers prepare these two foods with a BD of 3 and 4, respectively (Hackethal et al., 2023). In our study, AA levels of 558 μg/kg and 1030 μg/kg were measured in potato pancakes and 450 μg/kg and 1600 μg/kg in pan-fried potatoes with BD3 and BD4, respectively. The foods prepared with BD 4 exceed the benchmark levels set by the EC (750 μg/kg) in the food categories “Potato crisps from fresh potatoes and from potato dough” and “Other potato products from potato dough” and, therefore, it would be recommended to avoid BD4 in both foods (EC, 2017). Nevertheless, comparisons to the mentioned food categories are limited since pan-fried potatoes differ in their preparation to potato crisps and potato pancakes are not prepared only with potato dough. Due to the limitations of the present regulation, benchmark levels for foods such as potato pancakes and for other potato-based dishes are currently in discussion (EC, 2019). EFSA also reported high levels of AA in potato products (283 μg/kg - 606 μg/kg), with the highest levels measured in the category “other potato fried products”, which includes potato pancakes, potato pancakes

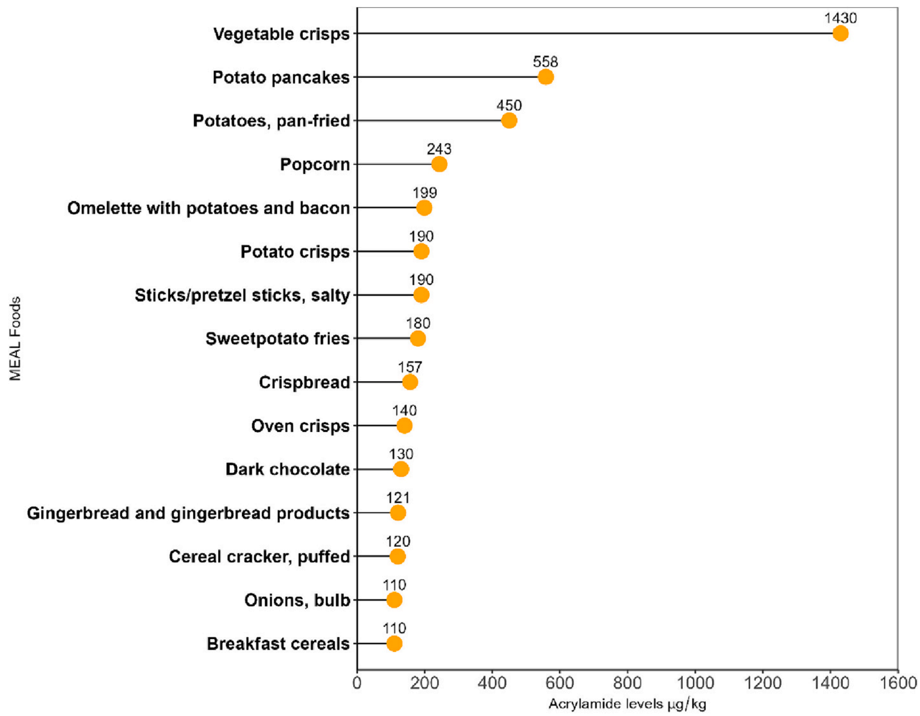

Fig. 1. 15 highest mean levels of acrylamide in the MEAL foods analysed (UB) [µg/kg]. If more than one preparation method or browning degree was investigated, the most preferred by consumers was used for the mean calculation (Hackethal et al. (2023).

and "rösti" (EFSA, 2015b). In the 2014 TDS from the United Kingdom, the highest levels of AA (465 µg/kg) were also measured in the category "potato crisps and potato-based snacks", containing similar foods as our pool "potato crisps" (190 µg/kg) (FSA, 2014; Hamlet, Andreou, & Carbone, 2015). Similarly, in the second French TDS, the highest AA levels were also found in potato products, such as potato crisps (954 µg/kg) and in the category "French fries and other fried potatoes" (724 µg/kg) (Sirot, Hommet, Tard, & Leblanc, 2012). They stated that the high AA levels in the French TDS in potato crisps compared to other studies could be due to pooling effects in TDS studies and that the use of different types of samples in TDS studies can lead to variations in results (Sirot et al., 2012). Furthermore, the results of the French TDS were published in 2012, before the EU regulation EC 2017/2158 benchmark levels for French fries were set, which may also explain the high levels of AA in the French study (EC, 2017). In 2021, Germany was the 6th largest potato producer country in the world and has been one of the countries with the highest consumption of potatoes since many years (FAO, 2021). Many traditional and popular dishes in Germany are prepared with potatoes, pointing out the importance of monitoring AA levels in potato-based products.

3.3. *French fries and sweet potato fries*

French fries and sweet potato fries were prepared using different cooking methods and BDs before testing for AA formation (Fig. 2). Overall, our results showed increasing AA levels with increasing BDs (Fig. 3), meaning that the longer the cooking time the more AA was formed. In French fries, AA levels increased fourteenfold from BD1 to BD2 in the air fryer. For sweet potato fries, there was a fivefold increase from BD2 to BD3 in the deep fryer. In addition, we observed that both foods had to be cooked longer to reach BD2 and BD3, which increased their internal temperature (Table 1). Other studies have shown similar results, when measuring the effects of time and temperature on AA formation (Gokmen, Palazoglu, & Senyuva, 2006; Truong et al., 2014). Although in our study there is a correlation between longer cooking times and high AA levels, it is also known that with increasing heat treatment, AA degradation processes as well as evaporation of AA from the food become more important. Thus, with increasing temperatures and time, AA levels in foods may decrease. This reduction is well documented, for example in coffee roasting, as longer roasted coffee, has less AA than lightly roasted coffee (Schouten, Tappi, & Romani, 2020). Nevertheless, longer cooking times are not always recommended as changes in taste and nutrient degradation have to be considered as well. The formation of AA in fries is a complex process, which can be influenced by several factors such as potato variety, storage temperature, moisture content and oil temperature (EC, 2017; Gokmen et al., 2006). Our results show that in the French fries prepared for BD3, independently from the cooking method, the levels of AA are three times higher than the benchmark level set by the European Commission at 500 µg/kg (EC, 2017). The MEAL surveys showed that German consumers have an overall preference for medium BDs (BD2 and BD3) in foods and dishes (Hackethal et al., 2023). However, as seen in the present study, foods such as French fries and sweet potato fries contained high levels of AA at BD3, and, therefore, should be avoided at this level.

In our current research, French fries developed more AA than sweet potato fries. This can be explained by the lower concentration of asparagine in sweet potatoes compared to white potatoes (Truong et al., 2014). In addition, the preparation of French fries and sweet potato fries varied slightly in our study, as it represents the consumer behaviour in Germany. For French fries, only pre-fried bagged potatoes were used, whereas for sweet potato fries, 40% of the subsamples were prepared with fresh potatoes and 60% with pre-fried bagged potatoes. This could have influenced the AA levels in the sweet potato fries, since the fresh sweet potatoes were soaked in water for one hour prior to preparation. As observed in other studies, soaking potatoes in cold water before preparing them can lower the content of AA in fries (Negoita, Mihai, & Hornet, 2022). Another reason for the lower AA levels in sweet potato fries in our study could be due to the addition of spices (e.g., garlic powder, rosemary) before frying. Other studies have shown that the use of rosemary and garlic powder can lower the AA content in fried potato products (Al-Anbari, Al-Musawi, Al-Ani, & Alkaraquly, 2019; Li et al., 2022). AA levels were measured in French fries and sweet potato fries

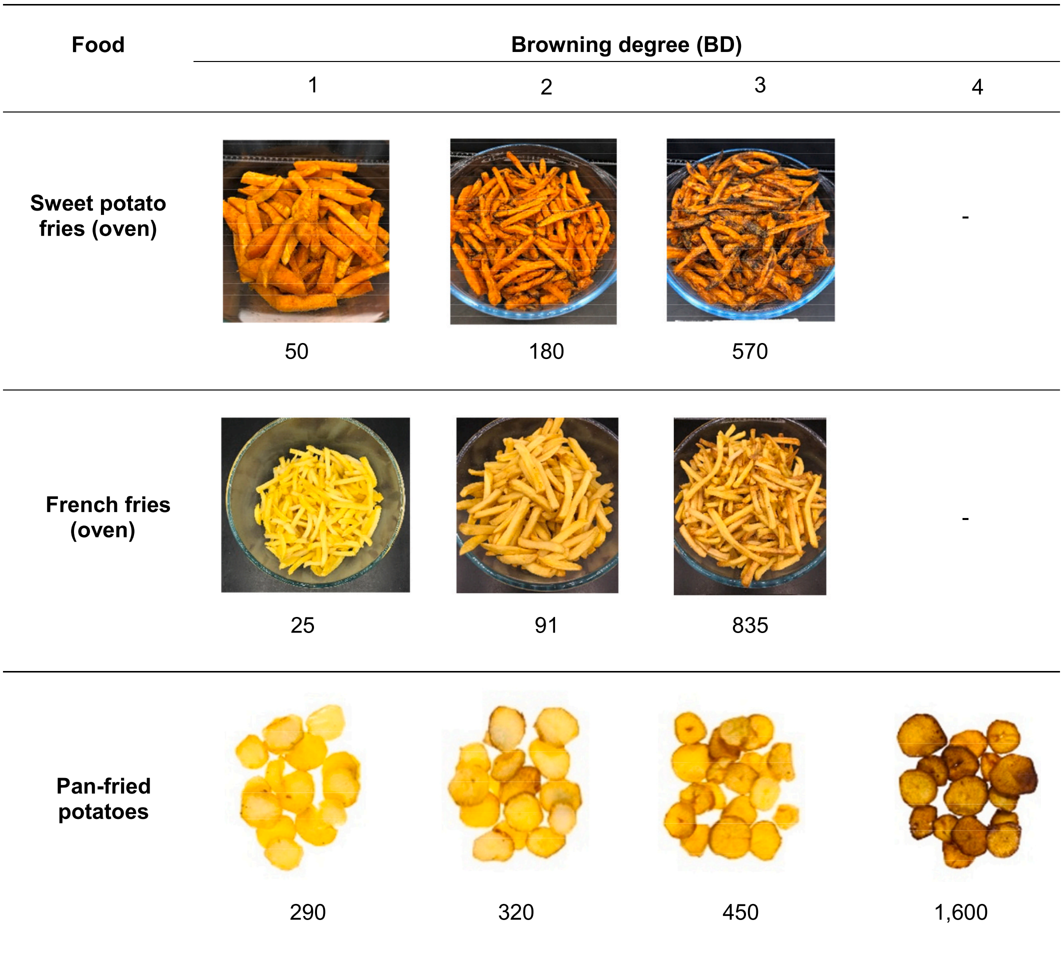

Fig. 2. Acrylamide levels (μg/kg) for sweet potato fries (oven), French fries (oven) and pan-fried potatoes prepared with browning degrees (1–4). Notes: sweet potato fries and French fries were only tested for BD 1, 2 and 3, as BD 4 and 5 were of no relevance in the consumer's surveys (Hackethal et al., 2023).

before preparation in our study kitchen (BD0) and both foods already showed low levels of AA (23 μg/kg and 20 μg/kg, respectively). This can be explained by the use of pre-fried bagged French fries and sweet potato fries in our samples. In addition to the BDs, AA levels were also examined using different cooking methods. For French fries prepared for BD1, the levels of AA were equally low for all three cooking methods. For preparation to BD2 and BD3, oven cooking was the method with the lowest levels of AA, while deep frying and air frying resulted in comparable higher levels. The levels of AA at BD3 prepared by air fryer and deep fryer were similar (1500 μg/kg and 1600 μg/kg, respectively). In contrast, a study by Dong et al. (2022) showed that air frying decreased significantly the amount of AA in French fries compared to deep frying. The slightly higher amounts of AA in deep frying could be due to the use of oil and the resulting oil oxidation, contributing to more AA formation (Dong et al., 2022). Based on our results, oven baking was the recommended cooking method for French fries. In sweet potato fries, the formation of AA prepared at BD1 and BD2 did not differ substantially between cooking methods (Fig. 3). Nevertheless, in BD3, preparation in the oven and in the deep fryer resulted in high levels of AA of 570 μg/kg and 1000 μg/kg, respectively. Even though literature on AA in sweet potato fries is more limited than for French fries, other studies have measured AA in sweet potato fries at a wide range of levels (136–3000 μg/kg) (Truong et al., 2014). Variations in AA levels between studies reflect differences in cooking temperatures, times and methods. Higher AA levels in sweet potato fries were observed at higher cooking temperatures, for longer times and/or when the frying oil was used several times. In our study, the goal was to achieve the colour/appearance for the different BDs based on consumer habits. To achieve the colour/appearance of the different BDs in the deep fryer, sweet potato fries had to be cooked longer than in other processing methods (Table 1), which may cause higher AA levels using this method. For sweet potato fries, a benchmark level has not yet been defined by the EC, although the EC, 2017 regulation recommends that food business operators should choose sweet potato varieties which are low in AA precursors (e.g. reducing sugars and asparagine) (EC, 2017). In summary, for French fries, the cooking method with the lowest AA levels was the oven, whereas for sweet potato fries the air fryer method resulted in less AA formation. The results from the MEAL surveys showed that most German consumers (60.6%) always prepare French fries in the oven, while 23.3% always prepare them in the deep fryer. As preparing French fries in the oven is a successful strategy to reduce AA levels in home-cooked French fries, consumers in Germany are encouraged to maintain this cooking habit.

3.4. Popcorn and salty sticks

Given the high temperatures at which salty sticks and popcorn are prepared, it is expected that AA is present in these foods. In our study, both foods contained AA, (220–280 μg/kg in popcorn and 190 μg/kg in salty sticks). In the present study, we tested different preparation methods of popcorn preparation (grill, microwave and stove) and the highest levels of AA were measured in the grilled popcorn (280 μg/kg). Although our measured AA contents for popcorn are in the range of values showed in other studies (106–761 μg/kg) (Zilic et al., 2022),

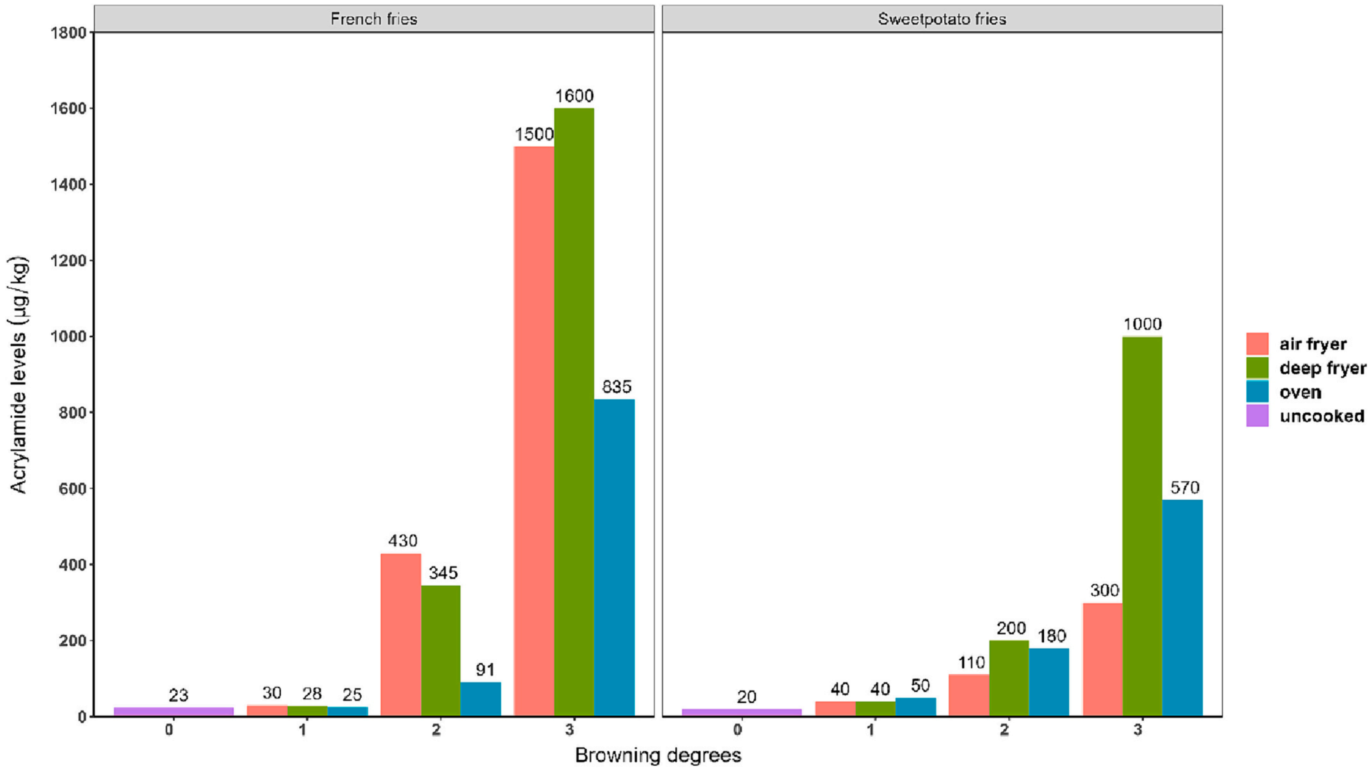

Fig. 3. Levels of acrylamide ([µg/kg]) in French fries and sweet potato fries prepared in air fryer, deep fryer and in oven with different browning degrees (1–3). Uncooked samples (BD 0) were also analysed, since pre-fried bagged French fries and sweet potato fries undergo pre-frying processes that can lead to the formation of AA at the factory level.

Table 1
Mean cooking times and maximal internal temperature measured in French fries and sweet potato fries prepared with different browning degrees and in air fryer, deep fryer and oven. Each cooking method and browning degree was tested 5 times per pool. The frying oil used to cook was renewed each time.

Browning Degree (BD)	Cooking method	Max temperature (°C)		Mean time (min.)	
		French fries	Sweet potato fries	French fries	Sweet potato fries
	Air fryer	78	73	9	7
	Deep fryer	92	80	3	12
BD1	Oven	72	70	5	6
	Air fryer	80	80	16	11
	Deep fryer	95	87	14	17
BD2	Oven	78	80	17	11
	Air fryer	97	98	32	15
	Deep fryer	96	97	21	27
BD3	Oven	98	98	34	17

preparation methods other than grilling (e.g. stove, microwave) should be prioritized by consumers and food producers. In Germany, the general per-capita consumption of snack food has increased over the last 10 years (Insights, 2023) and popcorn and salty sticks are two widely consumed snacks, especially among children and teenagers. A study in Poland found that 25% of teenagers consume >5 g of salty sticks per day, contributing to 2–5% of their daily AA intake (Wyka et al., 2015).

3.5. Dark chocolate

AA can be formed in cocoa beans during roasting, as this process requires high temperatures (100–160 °C). In our study, all chocolate samples contained AA. Due to the higher cocoa content, dark chocolate showed higher levels (130 µg/kg) compared to milk chocolate (32 µg/kg). Another study in Germany showed similar results, with higher amounts of cocoa beans (>50%) in chocolate leading to higher amounts of AA (Köppen, Rasenko, & Koch, 2015). Dark chocolate is known for its overall benefits when compared to milk chocolate in terms of higher satiety, lower energy intake and beneficial cardiovascular properties (Marsh, Green, Naylor, & Guelfi, 2017; Regecova, Jurkovicova, Babjakova, & Bernatova, 2020; Sorensen & Astrup, 2011). However, further studies are needed to understand the role of dark chocolate on AA exposure.

3.6. Coffee

Coffee was not among the foods with the highest AA levels in our study. All coffee beverages (including coffee brewed from automatic machine, French press, filter coffee and pad machines) had AA levels below the LOQ (20 µg/kg), with the exception of coffee prepared with the capsule machine and instant coffee, which had 21 µg/kg and 27 µg/kg of AA, respectively. The low levels of AA in coffee in our study are due to the use of brewed coffee, since water added to coffee powder dilutes AA considerably. Compared to our study, higher levels of AA (37 µg/kg) were reported in brewed coffee in the second French TDS (Sirot et al., 2012). According to Sirot et al. (2012), it is possible to estimate the levels of AA in dry coffee from coffee beverages given that the sample preparation is known. The estimation of AA levels is made by multiplying the quantity of AA found in coffee beverages (µg/kg) by the division between the quantity of water (g) and the amount of coffee used in the sample (g). In our study, we used a mean of 4 capsules (6,9 g of coffee/capsule) per 300 mL of water to prepare coffee in the capsule machine and for the preparation of instant coffee we used a mean of 20 g of coffee per 310 mL of water. Following the calculations of Sirot et al. (2012), in our study dried coffee used in the capsule machine contained 217 µg/kg of AA and instant coffee contained 419 µg/kg. These values are lower than the levels reported by EFSA in roasted coffee (dry) (249 µg/kg) and in instant coffee (dry) (1499 µg/kg) (EFSA, 2015b). In our study, we mostly used 100% Arabica coffee, while few samples were a mixture of Arabica and Robusta coffee. This may be a reason for the lower AA levels in coffee, since Arabica coffee contains lower levels of asparagine (AA precursor) than Robusta coffee (Schouten et al., 2020). Coffee is one of the largest contributors of AA exposure in adults, ranging from 3.1% to 24% of total exposure (EFSA, 2015b). In Germany, most consumers prepare coffee in filter coffee machines (44.4%), followed by automatic coffee machines (17.1%) and coffee pad machines (10.1%) (Hackethal et al., 2023). Coffee prepared with capsule machines and instant coffee, which showed the highest AA levels in our study, are preferred by only 7.4% and 5.6% of the German population, respectively (Hackethal et al., 2023).

Similarly to previous studies, we observed higher levels of AA in coffee substitutes (44 µg/kg) (Mojska & Gielecinska, 2013). Coffee substitutes used in our study were brewed with water and following packaging instructions (approx. 3 g of powder per 150 mL of water). If we apply the same calculations from Sirot et al. (2012), we estimate that dried coffee substitutes in our study contained 2200 µg/kg of AA, which is comparable to the levels reported by EFSA in coffee substitutes that contained chicory (2, 942 µg/kg) (EFSA, 2015b). Most of the coffee substitutes used in our study contained chicory. The high levels of asparagine in chicory are causing high levels of AA in coffee substitutes (Loaec et al., 2014).

3.7. Gingerbread and gingerbread products

In Europe, gingerbread and related products (e.g. spiced cookies), are very popular among adults and children, especially during the Christmas season. In our study, gingerbread and related products had lower levels of AA (121 µg/kg) compared to those reported elsewhere. In our study we tested industrial samples of gingerbread and its products only, and the standardized industrial processes might lead to lower AA levels compared to home-made products. High AA levels (<20 µg/kg - 8000 µg/kg) in these foods were described in Germany in the early 2000s (Amrein, Schonbachler, Escher, & Amado, 2005). These levels were similar to those measured in other European countries (EFSA, 2015b). This improvement may be a consequence of the implementation of the benchmark level in gingerbread (1000 µg/kg) by the EC (EC, 2017). Also, research conducted in Germany in recent years, has led to improvements in recipes and cooking methods for food producers and consumers. As an example, ammonium hydrogencarbonate (NH_4HCO_3) was shown to be a major contributor to high AA levels in gingerbread and should be replaced by sodium bicarbonate ($NaHCO_3$) (Amrein et al., 2005).

3.8. Further discussion

Compared to the results published by the German Federal Institute for Risk Assessment (BfR) in 2010, our current research reported overall lower levels of AA in foods in Germany. In 2010, high levels of AA were found in potato pancakes (692 µg/kg), soluble coffee (686 µg/kg), and in gingerbread ("Lebkuchen") and derived products (522 µg/kg) (EFSA, 2015b). Potato crisps and French fries had mean AA levels of 385 and 256 µg/kg, respectively (EFSA, 2015b). The overall decrease of AA levels/contents in foods in Germany may be due to the introduction and implementation of the ALARA ("As Low As Reasonably Achievable") principle in 2011. The ALARA principle states that AA levels in food should be as low as possible under the given production conditions and according to good agricultural practices. Therefore, food producers have been urged to minimize AA levels in highly contaminated product groups. At the same time, German consumers and restaurants were advised to follow cooking instructions and to avoid preparing food at high temperatures. The motto "*Vergolden statt Verkohlen*" ("baking golden brown instead of charring") was used as an awareness campaign to reduce the BD of foods (BfR, 2011). In our current study, we included many other foods that were not considered in 2010, such as vegetable crisps, popcorn, chocolate and sweet potato fries, which still contain high levels of AA. These results show the importance of conducting ongoing monitoring programs and the need to include a wide range of foods to be analysed for AA.

The results of the consumer surveys conducted within the MEAL study have recently been published and show different eating habits and choices in the German population (Hackethal et al., 2023). This study is an important complement to the present research, as it collects preferences for BDs and preparation methods relevant to AA formation. Interestingly, a high percentage of survey participants stated that they mostly consumed pan-fried potatoes (89.1%) and French fries (48.4%) at home versus out of home (Hackethal et al., 2023). Likewise, coffee and coffee specialities were mostly prepared at home by consumers (84.7% and 57.1%, respectively) (Hackethal et al., 2023). These foods are known to contain high levels of AA, and the fact that most consumers prepare them at home highlights the need to conduct studies that mimic consumer habits and preferences, especially in terms of cooking methods, BDs, ingredients and cooking utensils. In addition, these results highlight the importance of ongoing risk communication initiatives that inform consumers about best practices and preparation methods for foods (e.g. French fries) in order to avoid high AA levels in home-cooked foods. As stated in this research, consumers prefer BDs e.g., for French fries and sweet potato fries that contain high levels of AA.

Furthermore, survey results by Hackethal et al. (2023) showed that most consumers (58%) preferred consuming fried vegetables with BD 4. In the present study, vegetables prepared with different cooking techniques (e.g., frying) were tested for AA, and onion (110 µg/kg) and courgette (82 µg/kg) showed the highest AA levels compared to other vegetables (see Supplementary data, Table S3). It is known that vegetables also contain asparagine, and that exposure of asparagine-rich foods to temperatures above 120 °C can lead to the formation of AA. As the majority of consumers prepare vegetables with the appearance of BD4, further studies are recommended to evaluate the current exposure levels to AA through cooked vegetables in the German population.

Based on EFSA's dietary exposure results, infants, toddlers and other children are the most exposed group to AA. The main contributor to the total exposure of infants was baby food (infant formulae, fruit purée, ready-to-eat meal and dessert, excluding cereals) and the main contributor to the total exposure of toddlers, other children and adolescents was "Potato fried products (excluding potato crisps and snacks)" (EFSA, 2015b). Given the importance of baby food and potato-based products for AA exposure of infants and children, more studies are needed to understand the current levels of AA in these foods in children in Germany.

The MEAL foods containing high AA levels are similar to other European countries (EFSA, 2015b; Sirot et al., 2012). However, differences are observed in studies from other continents. In the sixth Chinese TDS, AA was mostly measured in the food groups "vegetables" and "cereals", and in Japan, the food groups "beverages" and "confectioneries" contributed most to AA intake (Kotemori et al., 2018; Zhu et al., 2022). Differences were also observed in South America, where, for example, "panela" (a product obtained by cooking sugarcane juice at high temperatures) and fried bananas are widely consumed and contribute to the AA intake (Cortes, Mejia, & Mahecha, 2021). These differences show that AA can be widely consumed in different food groups, and that AA consumption may vary from country to country depending on regional foods, eating habits, manufacturer-specific differences in industrial production and cooking methods. Due to the need to analyse specific foods and consumption patterns, TDSs are extremely relevant to inform risk assessors and managers. In recent years, several initiatives and campaigns in Europe (e.g., "AA Toolbox" by Food and Drink Europe and "goodfries.eu" by the **European Potato Processors' Association**) have alerted food operators and consumers to follow cooking practices that can minimize AA levels in food. These recommendations include videos explaining how to fry French fries, visual representations of BDs, recommendations on cooking temperatures, and how to handle frying oil during and after cooking. The publication of EU 2017/2158 (EC, 2017) was also a major step towards preventing the consumption of AA in Europe. In addition to the existing regulation, in 2019 the European Commission issued a recommendation for the monitoring of the presence of AA in other foods that were not previously included (EC, 2019). The extended list includes country-specific/regional foods such as “rösti” and “croquettes”, but also foods that have become widely consumed in Europe, such as “Mexican tortillas”, “pita bread” and “vegetable crisps”. Other items added to the EC list may seem surprising, such as for example “olives in brine”, but recent studies have found AA in olives which are heated at high temperatures (e.g., pizza with olives), and in black olives that are artificially coloured (BVL, 2021; Duedahl-Olesen et al., 2022). Based on our study, it would be advisable to include a benchmark for vegetable crisps due to their high AA levels. It was also demonstrated that some snacks, such as popcorn and salty sticks, have significant levels of AA, and therefore should be considered in further studies.

Although we measured lower levels of AA in food compared to 2010, a recent time-trend study showed that there was an increase in exposure levels of AA biomarkers (mercapturic acids of acrylamide (AAMA) and glycidamide (GAMA) in μg/L between 2001 and 2018 in the European population (Poteser et al., 2022). The same study mentioned that samples from German non-smokers contained higher levels of GAMA (13–23 μg/L) than other central/western countries. Exposure to AA in populations is mainly through smoke, water or diet. Given that non-smokers had increasing levels of AA biomarkers, Poteser et al. (2022) speculated that the current levels of AA biomarkers in the non-smoking population might be related to dietary exposure. Nevertheless, it is not clear if the current AA biomarker levels are due to increased levels of AA in foods or due to higher consumption of foods containing AA. In addition, Poteser et al. (2022) mentioned that regional differences regarding levels of AA biomarker levels may be due to ethnic differences in AA oxidation, which may contribute to some regions in Europe having higher levels of AA biomarkers than others. Further studies are needed to understand the current exposure pathways of AA in the German and European populations.

The establishment of a tolerable daily intake (TDI) for AA is controversially discussed in the scientific community. A recent study by Eisenbrand (2020) stated that there is insufficient evidence that AA causes malignant transformation in animal experiments, and therefore there is no reason to support a genotoxic mode of action (MOA). Instead, Eisenbrand (2020) proposed the establishment of a TDI for AA. In 2022, EFSA published an assessment on the genotoxic mode of action of AA and stated that the last EFSA opinion from 2015 would not be updated. In consequence, AA would continue to be considered as a genotoxic carcinogen (EFSA, 2015b; EFSA, 2022). Furthermore, EFSA added that the derivation of a TDI is not appropriate for substances considered to be genotoxic and carcinogenic.

3.8.1. *Study limitations and uncertainties*

The BfR MEAL study is a comprehensive TDS covering >90% of the foods consumed in German households, with a food list containing 356 foods. Nevertheless, the aggregation level of foods is lower compared to other TDSs. Due to the pooling of foods, no information is available on the levels of the substances in individual food samples. This uncertainty is accepted since the BfR MEAL study was designed to collect representative occurrence data and the obtained mean levels can be useful for further exposure assessment.

In the present study, brand loyalty was not taken into consideration. Foods purchased for the MEAL study included different brands and manufacturers in one pooled sample. This can be a limitation of our study since brand loyalty can be relevant for AA exposure. Exposure scenarios made by EFSA showed that when consumer’s preference for specific brands are taken into account a variation of 4% and 14% in potato fried products and in coffee, respectively, is observed when estimating AA exposure (EFSA, 2015b).

The UB and mLB approach used for the analysis of left-censored data may lead to over- or -under-interpretation of the mean values. The applied statistics have the following limitations typical for the TDS design: (i) it does not take into account that each MEAL pool already represents a mean level of the subsamples with an unknown variability, (ii) it may not be able to detect significant differences if there are significant differences in only some foods (iii) or if the direction of the differences is not the same.

The food consumption surveys VELS and NVS II on which the MEAL food list is based included age groups from 0.5 to <5 years and 14 to 80 years, respectively. Therefore, the dietary habits of children between the ages 5 to 13 were not included. Nevertheless, results by (Kolbaum, Ptok, Jung, Libuda, & Lindtner, 2023) showed that the resource-saving approach of considering only young children and adults in the MEAL study was sufficient to cover the food consumption habits of the middle groups as well. Since the consumption surveys were conducted in 2002 (VELS) and 2006 (NVS II), new foods and consumption trends emerged, which were not included in the present study. As shown by Kolbaum et al. (2023), new foods which may have high AA levels such as chicken nuggets/breaded chicken, veggie schnitzel and marshmallows could be added to the current food list. On the other hand, foods that contained high AA levels in our study such as gingerbread and gingerbread products, and omelette with potatoes and bacon are currently not as relevant as before and could be excluded from a future food list (Kolbaum et al., 2023). In future investigations, the updated food lists from the KiESEL and EsKiMo II studies should be used to reflect the diet of children (Brettschneider et al., 2018; Nowak, Diouf, Golsong, Höpfner, & Lindtner, 2022). Furthermore, consumption data were collected by 24-h recalls on three non-consecutive days for VELS and on two non-consecutive days for NVS II. The lack of a long-term record of food consumption may lead to underrepresentation of infrequently consumed foods.

Foods cooked for BDs were prepared with the goal of achieving the same appearance as the photos used for the BfR MEAL study consumer survey. Depending on the cooking method and the BD, cooking times varied and were not standardized. This approach is useful to imitate consumers’ behaviours but limits comparisons between cooking methods due to different cooking times used in the subsamples. In addition, the internal temperature of foods was measured after the foods were prepared, but the time of measurement was not recorded, leading to some uncertainty about potential temperature changes between the end of cooking and the time of measurement. Therefore, the decision was made to use the maximum internal temperature recorded as a reference, rather than the mean of the temperature in the subsamples.

4. Conclusion

Acrylamide levels for typically consumed foods were determined in the first German TDS. A total of 230 foods were analysed, providing a solid database for AA levels in Germany that can be used for further risk assessment studies. Due to their relevance for AA formation, 143 foods were prepared with different BDs and some foods such as French fries and sweet potatoes were prepared with different cooking methods (i.e. oven, deep fryer, air fryer).

Vegetable crisps had the highest mean AA levels, followed by potato pancakes and pan-fried potatoes. French fries and sweet potato fries also exhibited high levels of AA, especially when prepared at BD 2 and 3. For French fries, the cooking method with the lowest AA levels was the oven, while for sweet potato fries, the air fryer method resulted in less AA formation. AA was also found in snack foods such as popcorn and salty sticks. Coffee substitutes had higher AA levels than regular coffee and dark chocolate had higher levels than milk chocolate. Gingerbread and related products also contained some AA. For some relevant snack foods such as vegetable chips, popcorn, salty sticks and chocolates, the European Commission has not yet established a benchmark level for AA.

Since these foods had significant AA levels in our study, further investigations (i.e. exposure assessment) for AA in the German population would be recommended.

Due to the presence of AA in a wide variety of foods, it is advisable to test a broad range/spectrum of foods for this substance and to test different BDs and cooking methods that may affect AA formation. Mimicking consumer preferences and eating habits is crucial to understand the different pathways and potential sources of AA exposure in the population.

CRediT authorship contribution statement

Sara Perestrelo: Writing – original draft, Visualization, Validation, Investigation, Formal analysis. **Kristin Schwerbel:** Validation, Methodology. **Stefanie Hessel-Pras:** Conceptualization, Validation, Writing – original draft, Writing – review & editing. **Bernd Schäfer:** Validation, Writing – original draft, Writing – review & editing. **Martin Kaminski:** Conceptualization, Writing – review & editing. **Oliver Lindtner:** Conceptualization, Funding acquisition, Project administration, Writing – review & editing. **Irmela Sarvan:** Conceptualization, Methodology, Project administration, Writing – review & editing.

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

Data will be made available on request.

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Appendix A. Supplementary data

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