RELEVANCE OF DIETARY EXPOSURE TO ACRYLAMIDE FORMED IN HEAT-PROCESSED AGRI-FOOD PRODUCTS

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SUMMARY

Objectives: Acrylamide (AA) is considered one of the contaminants that occur in heat-processed agri-food products, which through diet can increase the risk of developing cancer for consumers of all age groups.

Methods: This review analysed the level of acrylamide of the most important heat-processed agri-food products that contribute to the dietary exposure of the population of different European countries and the assessment of health risks related to the presence of AA in food.

Results: The results of monitoring AA concentrations in agri-food products, reported individually by researchers or projects such as CONTAM in 2015 and the UK Food Standard Agency in 2017, show that some products exceeding the recently set European reference level are reported as such for specific values – mean UB/RLs in μg.kg^{-1}: French fries (550/500), coffee dry (523/400), coffee substitutes (1,499/500, 400), processed cereal-based baby foods (76/40), potato crisps and snacks (2,214/750), breakfast cereals (744/300), biscuits and crackers (637/350, 400), and coffee substitutes (1,897/500). Average values (μg/kg body weight per day) of exposure to AA from food for different age groups (EFSA) are estimated at 0.4–1.9, but in different European countries, as reported by several studies (including Romania), are between 1.4 and 3.4.

Conclusion: Starting from the genotoxic and carcinogenic potential of acrylamide, it is important to regularly monitor the presence of acrylamide and its levels in food and to investigate the food pattern of the population to detect the share of foods at risk of exposure.

Key words: acrylamide, monitoring level, vegetable food products, dietary acrylamide exposures, health risk assessment

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INTRODUCTION

The appearance of acrylamide (AA) or prop-2-enamide in a wide range of foods in relatively high concentrations has been reported since 2002 by the Swedish Food Authority. Using liquid chromatography-mass spectrometry, moderate levels of AA (5–50 μg.kg^{-1}) were detected in heated protein-rich foods, but higher contents of AA (150–4,000 μg.kg^{-1}) were detected in carbohydrate-rich foods (1). The mechanism of acrylamide formation has been associated with the processing of carbohydrate-rich foods based on carbohydrate-asparagine reaction under high temperature (usually > 120 °C) and low humidity conditions. Although the formation of the acrylamide in food has a dominant pathway through the reaction between asparagine and reducing sugars, four other types of chemical components as acrolein, acrylic acid, 3-aminopropionamide, and pyruvic acid are reported to have the potential to react with the amino groups available in the Maillard reaction (2).

The International Agency for Research on Cancer (IARC) classified AA as a carcinogen within the Group 2A (3). Animal testing has shown that acrylamide can be a health hazard to humans, has a genotoxic, neurotoxic and tumorigenic effect (4). In 2015, the European Food Safety Authority (5) published scientific opinion that acrylamide in food potentially increases the risk of developing cancer for consumers in all age groups and in 2017 the European Union has approved AA presence of acrylamide in food as carcinogenic agent (6). There are several initiatives focused on reducing acrylamide in heat-processed products from variety selection of storage, transport and processing control and educational resources for end-users regarding appropriate cooking practices: the guidance for industry on acrylamide in foods by the Food and Drug Administration (FDA) (7); mitigation measures and benchmark levels for the reduction of the presence of acrylamide in food established in 2017 by the European Commission; FoodDrinkEurope (FDE) in 2019 updated its Acrylamide Toolbox with new scientific and technological developments (8). It is well documented that the major sources of acrylamide from food of thermally processed agri-food are wheat, rye and potato products, biscuits, breakfast cereals, bread (especially fried), baked products, cakes, pies, French fries, crisps, and tasting products (9).

Exposure to food origin is an important part of the total exposure of the population to acrylamide. The main foods present in the diet of some western states contributing to AA exposure include potato chips (6–46%), French fries (16–30%), coffee (13–39%), breadstuffs and pastries (10–30%), and cookies (10–20%). In this context using the database at national, European and international level this paper evaluates the potential of dietary exposure to AA, presenting the evolution and current stage of AA levels in different thermally processed agro-food products, estimation of dietary exposure, and health risk assessment of acrylamide consumption.

MATERIALS AND METHODS

Monitoring Acrylamide Levels in Food

According to the recent EU regulation 2017/2158, the sampling and analysis of representative samples to monitor the levels of acrylamide is part of the evaluation of mitigation measures to reduce the presence of acrylamide in foods. In this context, the analysis method used to acrylamide concentrations must meet a set of performance criteria: Field blanks-less than limit of detection (LOD); repeatability (RSD) – 0.66 times RSDR; reproducibility (RSDR) – derived Horwitz equation (modified); recovery: 75–110%; limit of detection (LOD) – three tenths of LOQ; limit of quantification (LOQ): for benchmark level < 125 μg.kg^{-1}: ≤ two fifths of the benchmark level (however not required to be lower than 20 μg.kg^{-1}); for benchmark level ≥ 125 μg/kg: ≤ 50 μg.kg^{-1}.

The European Committee on Standardization (CEN) published a method by liquid chromatography coupled with electrospray ionization and tandem mass spectrometry (IL-ESI-MS-MS) for determination of AA in bakery products like bread, toasted bread, crisp bread, butter cookies, and biscuits, as well as potato products and roasted coffee by gas-chromatography mass spectrometry (GC-MS), CEN/TS 2017 method (10).

RESULTS

Acrylamide Levels in Popular Foods

The presence of acrylamide in food on the European continent was collected after 2003, starting with the Institute of the Joint Research Centre for Reference Materials and Measures, and since 2007 by Member States as data providers reported by the European Food Safety Authority. The report of the 2015 CONTAM panel, based on data provided by the Member States and six food associations, shows that the highest levels of acrylamide were detected in vegetable crisps and coffee substitutes (Fig. 1).

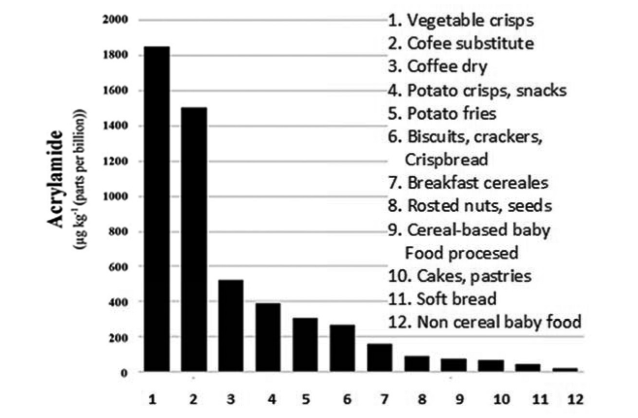

Fig. 1. *Mean acrylamide levels in selected food groups or products.*

Data from EFSA (5) on contaminants in the food chain

The highest levels of acrylamide were recorded at chicory coffee substitute (average value was 2,942 μg.kg^{-1}, 95th percentile was 4,500 μg.kg^{-1}); for dry coffee, which is consumed in larger quantities, average was 522 μg.kg^{-1}. For other popular foods, acrylamide levels have hundreds of μg.kg^{-1}, which explains the concern for food supply as these levels exceed the tolerance levels set for drinking water and a wide range of basic foods are affected (9).

The study on acrylamide content in food commodities consumed during 2018 from the markets, bakeries, restaurants, and fast food premises in North Macedonia (11) presents average values/range (μg.kg^{-1}) for a number of basic foods: biscuits, wafers, cakes (231.1 ± 191.7, range < 50–666.8); crisps (252.0 ± 176.4, < 50–788.6); breakfast cereals (146.5 ± 89.7, < 50–305.6); French fries (494.5 ± 127.1, range < 214.3–657.4); and potato crisps (390.6 ± 192, range < 168.4–861.0). It is noted that interval values for all products concerned exceed the indicative levels according to the EU Commission Regulation 2158/2017 (6) with percentages between 5.0% for breakfast cereals up to 50% for potato crisps.

A recent study conducted in February 2019 for determining the level of AA in 70 classical potato crisps samples marketed by 33 producers in Spain has as objective the updating of the global situation in the snack sector (12), and the evaluation of effectiveness of the mitigation strategies established by the European Commission Regulation 2017/2158. The obtained results show that the concentrations of AA in all samples vary between 89 to 1,930 μg.kg^{-1}, and the mean, median and 95th percentile values were 664 μg.kg^{-1}, 569 μg.kg^{-1} and 1576 μg.kg^{-1}, respectively; the values determined are higher than those reported by EFSA (5) in European countries for the category of potatoes obtained from fresh potatoes (average value: 392 μg.kg^{-1}, 95th percentile: 949 μg.kg^{-1}, n = 31,467).

However, the results obtained from a series of studies are within the limits of the results reported in recent years in a number of countries: potato chips from Hanoi (Vietnam) have values between 25 and 1,620 μg.kg^{-1} (13); average value of 642 μg.kg^{-1} in potato crisps from Cyprus (10–2193 μg.kg^{-1}) (14); higher concentrations were reported in potato crisps in Italy (15) with values of 3.444 μg.kg^{-1} and 162 μg.kg^{-1}); lower value for potato crisps were reported from Portuguese local markets, with average value 475 (16).

Another study on the concentration of acrylamide in French fries prepared in school canteens from different Spanish regions (17) reported an average content of 329 μg.kg^{-1} (< 20 to 4000 μg.kg^{-1}). The acrylamide content of fried potatoes prepared from the frozen product (229 μg.kg^{-1}) is lower than in fresh potatoes 460 μg.kg^{-1}, and only 15.7% of the samples exceeded the reference levels established by the EU Regulation 2017/2158 (500 μg.kg^{-1}). It is confirmed the directly proportional relationship of the determined acrylamide concentrations with the colour of toasted (2,274 μg.kg^{-1}), dark-golden (463 μg.kg^{-1}), gold (134 μg.kg^{-1}), and light-golden (52 μg.kg^{-1}) French fries.

Recent institutional project EFSA-CONTAM Panel (5) and the UK Food Standard Agency report the results of acrylamide levels in a range of retail foods and offer the possibility of reporting concentrations determined within these large-scale projects at the reference levels (RLs) recently established by the EU Commission Regulation 2158/2017, (Table 1). The levels of AA determined and reported in Table1 show that for some products the recently established AA reference levels were exceeded. Examples of

Table 1. *Acrylamide levels in agro-food products according to European projects*

Food category	Acrylamide (µg.kg^{-1})						
	CONTAM Panel EFSA (2015)			FSA-UK*			CR 2158 EU (2017)
	Mean MB	Mean LB	Mean UB	Mean	Min	Max	RLs
French fries sold ready to eat	156	21	550	308	302	314	500
Potato crisps and snacks	389	388	389	631	25	2,214	750
Soft bread	42	36	49	23	3	96	50, 100
Breakfast cereals	161	157	164	221	33	744	300
Biscuits, crackers, crisp bread and similar	265	261	269	207	34	637	350, 400 350
Coffee (dry)	522	521	523	131	94	164	400
Instant coffee (dry)	–	–	710	504	312	641	850
Coffee substitutes (dry)	–	–	1,499	818	237	1,897	500[a], 400[b]
Processed cereal-based baby foods	73	70	76	17	2	58	40
Other products, based cereals, potatoes, cocoa and coffee	97	92	101	132	4	326	150

*Source: Hamlet et al. (31)
FSA – Food Standards Agency; CR – commission regulation (EU 2017)
Mean MB (LB-UB): MB – middle bound, estimate; LB – lower bound, estimate; UB – upper bound, estimate; RLs – reference levels (Commission Regulation 2017/2158);
[a]coffee substitutes exclusively from cereals; [b]coffee substitutes exclusively from chicory

concentrations (reported/RLs) µg.kg^{-1}: CONTAM project for products such as French fries (550/500), coffee dry (523/400), coffee substitutes (1,499/500a, 400b), processed cereal-based baby foods (76/40); the UK project for products such as potato crisps and snacks (2,214/750), breakfast cereals (744/300), biscuits and crackers (637/350, 400), and coffee substitutes (1,897/500).

In this context, in 2019 FoodDrinkEurope – a set of tools (toolbox) was developed, a framework aimed at acrylamide formation pathways and mitigation strategies (8).

In Romania, little is known about the AA level of heat-processed agro-food products and the dietary exposure of the population to this contaminant (22). A very recent study, conducted in 2018–2019 on 46 samples of cereal-based food products, showed that in all samples were detected AA levels in the range of 5.63 µg.kg^{-1} to 548.80 µg.kg^{-1}, and a percentage of 8.7% of the total samples exceeds the limit level set by the EU Regulation 2017/2158 (6), the highest level of AA was determined in 4 biscuit samples exceeding the reference level provided by the European Union (18).

Estimates of Dietary Acrylamide Exposures

The detection and level of AA in heat-processed foods is a concern for the health of the population as this substance has been classified by the IARC (3) as probable carcinogenic to humans.

Assessment of dietary exposure and daily dietary intake of AA by food category was based on equations (19):

Dietary exposure = Σ (daily food intake × chemical concentration in food)

$$DI_{AA} = (DFI \times C){:}1000$$

where DI_{AA} is the daily AA intake (µg/day); DFI is the individual mean daily food intake (g/day); C_{AA} is the mean AA concentration per food item in (µg.kg^{-1}:1000) for converting kg in g of food.

A number of international bodies have estimated dietary exposure to AA and carried out risk assessments related to the presence of AA in foods since 2002. The highest estimates were reported by the Joint Expert Committee on Food Additives (JECFA), which in 2005 concluded that the AA estimates of dietary exposure of consumers were 1 µg/kg body weight/day, average, and 4 µg/kg bw/day for a high percentage of distribution (20). These estimates were updated in 2010 using data sent to JECFA and taken from the literature, and using the Global Environment Monitoring System (GEMS) food data – Brazil, China, France, Ireland, New Zealand, Norway, Spain, and the United States provided information on the occurrence of AA and dietary exposure. Thus, national estimates of dietary exposure to acrylamide in foods such as crisps and French fries, breads and rolls, breads, and sweet biscuits (cakes) ranged from 0.2 to 1 µg/kg bw/day for the general population, including children, and from 0.6 to 1.8 µg/kg bw/day, in the percentiles from 95 to 97.5. In conclusion, there was no change in dietary exposure to AA between the 2005 and 2010 assessments, JEFCA maintained the value of 1 µg/kg bw/day and 4 µg/kg bw/day estimates for safety assessment purpose (22).

The reports of CONTAM Group (5) estimate the contribution of different foods to food intake in different EU member states and acrylamide exposure levels across the different population groups, infants, toddlers and other children. According to this project, the average exposure levels have varied from 0.6, minimum LB (lower bound) to 1.9 µg/kg bw/day maximum UB (upper bound), and 95th percentile from 1.4 (minimum LB, estimate) to 3.4 µg/kg bw/day (maximum UB, estimate) depending on the survey and age group. The Environmental Protection Agency in the USA (US EPA) proposes the reference dose value <0.002 mg/kg bw/day for oral intake of acrylamide (23).

According to EFSA scientific opinion (5), the margin of exposure (MOE) for the carcinogenic effects of AA indicating a concern for public health, corresponding to the ratio between benchmark dose lower confidence limit (BMDL10) and dietary exposure of the population, ranges from 425 for average adult consumers to 50 for young children. Experts cited by EFSA in 2015 proposed two BMDL10s for AA at 0.17 μg/kg bw/day for neoplastic effects in mice and 0.43 μg/kg bw/day for peripheral neuropathy in rats. Exposure range MOE for AA effects on cancer, corresponding to the ratio of lower 95% confidence limit BMDL10 to dietary exposure of the population, ranges from 425–50 for middle-aged and low-intake children.

The Food and Drug Administration (7) analysing acrylamide occurrence data and exposure estimates for 2011–2015 found that the largest contributors to food intake of acrylamide continue to be French fries and potato products, breakfast cereal, cookies, potato chips, and crackers. Mean dietary intake for those 2 years old and older based on 2011–2015 data was 0.36 μg/kg bw/day, comparable to 0.44 μg/kg bw/day reported by FDA (24), while CONTAM Panel estimated the mean exposure between 0.4 and 1.9 μg/kg bw/day, across all age groups, with the 95th percentile at 0.6 to 3.4 μg/kg bw/day (5).

The level of exposure to acrylamide from potato crisps in the Spanish population (17) ranges between 0.33 and 7.09 μg/person/day, with a mean value of 2.44 μg/person/day. Considering total data per capita consumption of this foodstuff at 1.34 kg/person/year, mean daily intake of acrylamide from potato crisps for the Spanish population was estimated to be 0.035 μg/kg bw/day, ranging from 0.005 to 0.101 μg/kg bw/day, similar to that observed for other countries in studies with different population groups.

The study from North Macedonia on acrylamide content in food commodities consumed and its risk assessment in the population reports mean AA levels of 126.9 ± 122.4 $\mu g.kg^{-1}$ for bread samples and 494.5 ± 127.1 $\mu g.kg^{-1}$ for fried potato samples, so the dietary exposure of the population to AA was calculated: 0.643 ± 0.171 μg/kg bw/day (11). In this study, bread was found to be the main contributor to total AA intake with an estimated value of 0.394 ± 0.150 μg/kg bw/day with a margin of exposure values calculated on average consumption showing 528 for neurotoxicity and 264 for non-plastic effect, and the 95th percentile was 242 for neurotoxicity and 121 for neoplastic effects. According to EFSA (5) for the non-genotoxic compound, MOE value of 100 is considered a safe value so there is no health risk.

A cross-sectional descriptive study on AA levels in traditional foods and the assessment of AA dietary exposure conducted in 2018 in the Lesser Poland region reflected the general average values and the 95th percentile proved to be 0.213 and 0.458 μg/kg bw/day, respectively (25).

The cross-sectional study, conducted among children in Romania kindergartens, estimated the daily intake of acrylamide (AA) in a community of 78 children aged 4 to 6 years. Daily average consumption during the survey period for each child resulted from the ratio between the sums of the values obtained for each day/person and the number of days investigated (26). AA levels in food taken into account in this study come from the EFSA database (5) including 206 samples from Romania, and from data reported by FAO/WHO 2011 (22) for 31 countries, including 22 countries in Central and Eastern Europe. Based on the average concentrations of acrylamide in food and the average amount of food consumed, mean of total daily intake exposure to acrylamide (TDIAA) was 2.2 μg/kg bw/day, while the correspondingly high exposure in high percentages 95 to 97.5 was 7.26 μg/kg bw/day (26). The national values obtained in this study (7.26 μg/kg bw/day) AA, exceed the baseline estimates reported by the EFSA (5) at 1.4 and 3.4 μg/kg bw per day. The main foods that contributed to the average level of total food exposure were cereals (31.1%) and vegetables (30.8% of total exposure), the potato being the major contributor (13.9% of total exposure). Average values and high percentile distribution of TDIAA% expression distribution are illustrated in Figure 2 (26).

The chronic exposure to AA in bread and biscuits of the Romanian population was estimated based on a study conducted on 19 samples of commercial bread and 13 samples of biscuits and wafers category (18). The calculations took into account per capita consumption and the average level of AA of these products according to FRD 2016 reports (27). The level of exposure of the population to AA in bread of 0.9 μg/kg bw/day is much higher than the levels in some European countries: 0.01 and 0.05 μg/kg bw/day in Poland (25); 0.21 μg/kg bw/day for toast and bread products in Slovenia (28); levels of 0.020 and 0.024 μg/kg bw/day, respectively, among children and adults in France (20).

The biscuits and wafers category in the Romanian markets also contributes to the chronic exposure to AA of consumers with average values also 0.9 μg/kg bw/day (27), value located at the upper limit of the chronic exposure interval reported by EFSA (5) (0.4–0.9 $\mu g.kg^{-1}$ bw/day) and well above the exposure values reported by some European countries. Thus Slovenia reports an exposure level of 0.03–0.04 $\mu g.kg^{-1}$ bw/day for biscuit and wafer consumption (28), and France reports a level (μg/kg bw/day) of 0.017 for sweet biscuits for adults and 0.075 for children (29).

DISCUSSION

Health Risk Assessment

The risk assessment, including the assessment of dietary exposure, provides the scientific basis for setting standards, guidelines and other recommendations of international institutions with responsibilities in the field of food safety. This ensures that food

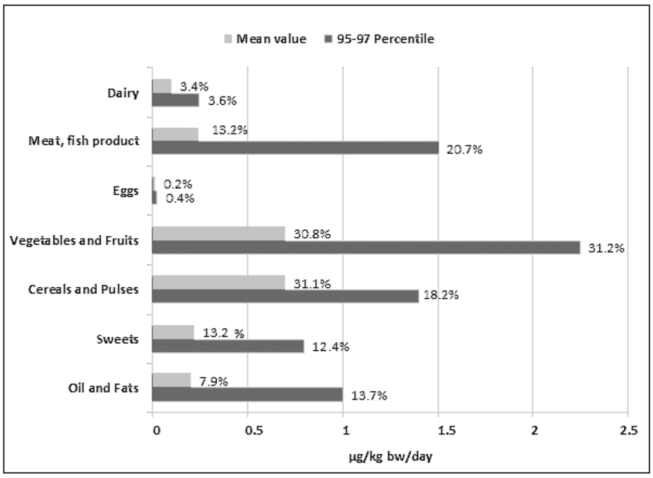

***Fig. 2.** Group of foods that contribute to estimation of the exposure to acrylamide (26)*

Mean values and the high percentile distribution (PT 95-97) of the TDIAA% expression-distribution

Table 2. *Daily acrylamide intake hazard quotient values**

	HQ		ADD		HQ_t		ADD_t	
	Male	Female	Male	Female	Male	Female	Male	Female
Coffee beans	0.19	0.24	0.01	0.24				
Potato chips	0.02	0.03	0.01	0.01	0.55	0.70	0.28	0.35
French fries	0.35	0.43	0.17	0.22				

*Source: Oroian et al. (32)
HQ – hazard quotient; ADD – average daily acrylamide intake (μg/kg bw/day); HQ_t – total hazard quotient; ADD_t – total average daily acrylamide intake

safety requirements protecting public health are consistent across countries and are suitable for use in international trade.

In agreement with the US EPA standard methods (23), the risk of chronic-toxic effects is expressed as the ratio of the dose resulting from exposure to the environment of dose considered for safety, even to sensitive persons. This ratio called the hazard coefficient (HQ) applied to the assessment of the risk to human health through the chronic effects of acrylamide consumption was estimated using the following calculation formulas:

$$HQ = ADD/RfD$$
$$ADD = (C \times IR)/BW$$

where ADD is the average daily acrylamide intake (μg/kg bw/day), RfD is the daily intake reference dose (μg/kg bw/day) recommended by the European regulations, C is the mean acrylamide concentration in food ($\mu g.kg^{-1}$), IR is the coffee, potato chips and French fries rate (kg/person/day), and BW is the average body weight (kg bw).

The total hazard coefficient (HQ_t) indices are used to estimate the total chronic-toxic risks of acrylamide according to the number of toxicity sources studied:

$$HQ_t = HQ_1 + HQ_2 + HQ_3$$

The indicator values for all analysed chemicals and (HQ_t), (Table 2) are multiplied by 10^{-6} to estimate the cumulative risk of exposure to carcinogens:

$$Risk = (HQ_t) \times 10^{-6}$$

HQ values lower than 1 are considered as no significant risk of chronic-toxic effects. A risk greater than 10^{-6} for any individual carcinogen or greater than 10^{-5} for the sum of all carcinogens may require further evaluation.

According to the CONTAM Panel scientific opinion, coffee beans, potato chips and fried potatoes have the highest levels of acrylamide. The study of the risk assessment for health, through the consumption of products from different producers on the Romanian market using the HPLC-DAD method, reported the quantified acrylamide levels ($\mu g.kg^{-1}$, mean value) in the grains coffee (1,115), potato chips (600) and French fries (783). These data was used to estimate daily intake, hazard ratio, cumulative risk and carcinogen to express potential health risks (30).

The total hazard coefficient indices are used to estimate the total chronic-toxic risks of acrylamide according to the number of toxicity sources studied, in this case coffee, potato chips, and French fries (Table 2).

It is noted that HQ determined in this study has subunit values (0.02 of potato chips for males and 0.43 of French fries for females), and HQ_t values for males and females are lower than 1 (varying in potato chips between 0.70 for females and 0.55 for males). Thus, based on these obtained values, it can be estimated that the contribution of acrylamide through the consumption of coffee, potato chips and fried potatoes did not have a significant potential for chronic-toxic risk for Romanian consumers.

CONCLUSIONS

Starting from the genotoxic and carcinogenic potential of acrylamide, it is important to regularly monitor the presence of acrylamide and its levels in food, investigate the dietary pattern of the population to detect the share of food and the risk of exposure, and to formulate strategic directions for reducing human exposure to acrylamide. The results presented in this review confirm the general recommendation for consumers to raise awareness regarding food selection and food preparation techniques, a healthy balanced diet and limits of the amount of baked cereal and fried products for all age groups to reduce their exposure to acrylamide.

Conflict of Interests

None declared

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