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Acrylamide Content in Food Commodities Consumed in North Macedonia and Its Risk Assessment in the Population

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HIGHLIGHTS

- Mean acrylamide levels varied from 126.9±122.4 µg/kg for bread samples to 494.5±127.1 µg/kg for French fries samples.
- Acrylamide dietary exposure of the population for the tested foods was estimated at 0.643±0.171 µgAA/kg$_{bw}$/day.
- Monitoring programs and mitigation strategies of acrylamide in foodstuffs must be implemented in North Macedonia.

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Acronyms and abbreviations
AA=Acrylamide
MOE=Margin of Exposure
BMDL$_{10}$=Bench Mark Dose Lower
MS=Mass Spectrometry
LC=Liquid Chromatography
GC=Gas Chromatography
UHPLC=Ultra High Performance Liquid Chromatograph
QuEChERS=Quick Easy Cheap Effective Rugged Safe
LOQ=Limit of Quantification
MRM=Multiple Reaction Mode

ABSTRACT

Background: Acrylamide (AA) is an important food contaminant resulted from Maillard reaction during thermal processing of carbohydrate rich food commodities. The present paper reports the data for the AA content in some types of thermally processed starch rich food, and assessment of dietary exposure for the population in North Macedonia.

Methods: The AA level was determined employing modified and validated ultra high performance liquid chromatography with tandem quadrupole detector. A total of 160 samples divided in seven most frequently consumed commodity groups were collected for determination of their AA content. Finally, chronic exposure of AA in the population was estimated. Statistical analysis was performed applying OriginPro 8 SR4 v8.0951 software package

Results: The average AA levels varied from 126.9±122.4 µg/kg for bread samples to 494.5±127.1 µg/kg for French fries samples. The dietary exposure of the population from North Macedonia for the tested food commodities was estimated at 0.643±0.171 µg$_{AA}$/kg$_{bw}$/day. The main contributor to the total AA intake was bread, with estimated value at 0.394±0.150 µg$_{AA}$/kg$_{bw}$/day. The margin of exposure values were 528 and 264, respectively for neurotoxicity and non-plastic effect calculated on average intake.

Conclusion: The risk assessment analysis revealed increased concern for human health regarding the neoplastic effects, especially for infants, toddlers, and adolescents. This is the first study related to AA presence in different food commodities in North Macedonia, and implies that monitoring programs and mitigation strategies must be implemented.

Introduction

Acrylamide (AA) is an important food contaminant resulted from Maillard reaction during thermal processing of carbohydrate rich food commodities. Specifically, it is determined that AA is a result of the reaction

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between amino acid asparagine and reducing sugars, and their presence and amount in some types of food is considered as main precursor for AA formation to high extent (Friedman, 2003; Keramat et al., 2011; Mesias and Morales, 2016). Historically, AA was thought to be a contaminant present in the water, and its exposure to humans has not been a matter of high concern (Mastovska and Lehotay, 2006). However, since 2002 when the Swedish National Administration revealed the first results from AA analysis in some food types, AA became an issue that attracted the scientific community (Kim et al., 2007). The International Agency for Research on Cancer (IARC, 1994) classified AA as a probable human carcinogen (group 2A), given the evidence in animal studies for potential carcinogenicity for the HEK293 cell line (Celik et al., 2018).

The tolerable daily intake of AA was determined to be 40 μg/kg$_{bw}$/day for neurotoxicity, and 2.6 μg/kg$_{bw}$/day for carcinogenicity (Tardiff et al., 2010). According to EFSA (2015) the risk characterization for peripheral neurotoxicity could be carried out by the Margin of Exposure (MOE) approach as well as the Bench Mark Dose Lower confidence limit for 10% change in the response rate (BMDL$_{10}$) value of 430 μg/kg$_{bw}$/day. For risk characterization for neoplastic effects, the MOE approach for compounds is considered effective, by the BMDL$_{10}$ data of 170 μg/kg$_{bw}$/day, that are both genotoxic and carcinogenic.

The detected levels in food are a result of complex and competitive processes of AA formation and elimination, or degradation (Claeys et al., 2010). It has been found that the largest amount of AA is accumulated during the last backing, roasting, or frying stages in which the moisture content of food products decreases, with exception of coffee in which the AA level decreases during the last stage of roasting. The main sources of AA in the diet are potato products such as French fries and potato crisps, cereal products such as breakfast cereals, bread, biscuits, cookies, various starch rich crisps, and roasted coffee as well as coffee substitutes (Mesias and Morales, 2016; Vinci et al., 2012). Due to multiple sources of dietary exposure to AA, the Food and Agriculture Organization/World Health Organization (FAO/WHO) considered that 1 μg/kg$_{bw}$/day is an average exposure to AA (JECFA, 2006). The exposure of 4 μg/kg$_{bw}$/day is considered as high exposure level to AA with, and a mean dietary exposure range is 0.4-0.8 μg/kg$_{bw}$/day for the general adult population, while the calculated 95th percentile exposure is 0.6-1.8 μg/kg$_{bw}$/day (JECFA, 2011).

Since AA was identified as serious threat for human health and intensive monitoring measures were needed to be conducted, many studies have proposed suitable analytical methods to detect this compound in variety of food samples. Many different approaches were investigated but mostly the proposed methods were those using Mass Spectrometry (MS), either coupled to Gas Chromatography (GC) (Kawata et al., 2001; Mastovska and Lehotay, 2006; Ono et al., 2003; Tareke et al., 2002) or Liquid Chromatography (LC) (Chen et al., 2012; Claeys et al., 2010; Kim et al., 2007; Mastovska and Lehotay, 2006; Roach et al., 2003). MS was applied either in selected ion monitoring mode, or, by tandem Mass Spectrometry (MS/MS) in Multiple Reaction Mode (MRM), using isotope labeled AA standard. The GC-MS methods might have a drawback, because in some cases a derivatization step of AA is needed prior to the sample injection. So, LC-MS or LC-MS/MS is more frequently used for AA analysis. Regarding the extraction, Solid Phase Extraction (SPE) was widely used, and lately, QuEChERS (Quick Easy Cheap Effective Rugged Safe) procedure was introduced based on matrix-dispersive sample preparation (Mastovska and Lehotay, 2006).

So far, there are no data for the present of AA levels in food marketed in North Macedonia, and consequently the exposure of the population is unknown. This study reports the data for the AA content in some types of thermally processed starch rich food, and assessment of dietary exposure for the population in North Macedonia.

Materials and methods

Sample collection and preparation

For this research, food samples assumed to contain high AA levels were collected during 2018 from the markets, bakeries, restaurants, and fast food objects located in North Macedonia. In total, 160 samples were analyzed, among which, 33 were bread samples (white and whole grain bread), 22 pastry samples, 20 breakfast cereals, 25 salted crackers, 14 French fries, 29 potato crisps, and 27 biscuits and baked cakes. Two hundred g of the samples were homogenized and kept at temperature below -18 ^{o}C until analyzed.

Chemicals

Acrylamide (purity≥99%) and acrylamide-d$_3$ (0.5 mg/ml solution in acetonitrile) standards, and formic acid were supplied from Sigma Aldrich (Diesenhofen, Germany). Acetonitrile gradient grade, acetonitrile MS grade, *n*-hexane and water MS grade were supplied from Carlo Erba (Milano, Italy). The QuEChERS extraction kit (mixture of 4 g of magnesium sulfate and 1 g of sodium chloride) and matrix-dispersive clean-up kit (mixture of 900 mg magnesium sulfate, 150 mg primary secondary amine and 150 mg C18 bulk phase) were produced by Scharlau, Scharlab (Barcelona, Spain).

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Analytical instrument and MS conditions

The samples were analyzed by modified and optimized LC-MS/MS method with previous sample preparation applying modified QuEChERS procedure proposed by Mastovska and Lehotay (2006). Waters (Milford, MA, USA) system-acquity Ultra High Performance Liquid Chromatograph (UHPLC) equipped with binary pump, autosampler and column thermostat, coupled to tandem quadrupole detector was used for UHPLC-MS/MS analysis of AA. For analytical separation, ZORBAX SB Aq column (50 mm x 2.1 mm x 1.8 µm) product was used from Agilent Technologies (Santa Clara, CA, USA). In addition, the chromatographic separation was performed at flow rate of 0.15 ml/min, column temperature 25 ^{o}C, at isocratic conditions with mobile phase composition 0.1% formic acid/methanol (95:5). Total run time was 8 min. The MS determination was performed with electro-spray ionization in positive mode (ESI+), nitrogen as desolvation gas (500 L/h) and cone gas (50 L/h). Hence, the source and desolvation temperature were set at 150 and 400 ^{o}C, respectively. MRM traces were acquired with the characteristic fragmentation transitions shown in Table 1. Furthermore, Data acquisition and processing was performed with MassLynx software version 4.1 product of Waters (Milford, MA, USA).

Preparation of standards

Acrylamide stock solution with concentration 1 mg/ml of prepared in methanol, and acrylamide-d_3 stock solution with concentration 0.5 mg/ml were kept at less than -18 ^{o}C. Applying a suitable dilution, six working calibration standard solutions for AA with concentrations of 10, 50, 100, 200, 500, and 1000 ng/ml were prepared in 0.1% formic acid. A standard solution of acrylamide-d3 with concentration of 1000 ng/ml was used for sample spiking at different concentration levels, in accordance with the validation experiment.

Sample preparation

A portion of 2±0.01 g homogenized sample was weight in 50 ml plastic polyethylene tube. All the samples were analyzed in duplicates. Four hundred µl of acrylamide-d_3 spiking solution (equal to level of 200 ng/g) was added to each sample. Five ml of *n*-hexane were added with pipette and the tube was sealed and vortexed for 1 min. Afterwards, 10 ml of deionozed water, 10 ml acetonitrile, and extraction mixture of magnesium sulfate and sodium chloride were added, and the sealed tube was immediately vortexed for 1 min, avoiding production of crystalline agglomerates, and also providing sufficient solvent interaction with the entire matrix. The tube was centrifuged at 4500/rpm for 10 min at room temperature. The upper *n*-hexane layer was carefully discarded by aspiration with plastic Pasteur pipette, and 6 ml of the acetonitrile layer were transferred into 15 ml plastic tubes containing mixture of 900 mg magnesium sulfate, 150 mg primary secondary amine, and 150 mg C18. The tube was sealed, vortexed 1 min, and centrifuged at 4000/rpm for 5 min at room temperature. Two ml of the supernatant were transferred into autosampler vial and 20 µl of 10% formic acid were added to facilitate the analyte ionization. With this procedure, the dilution factor for the samples was 5. For bread and breakfast cereals samples in which the expected AA levels were lower, we applied two-fold concentration by evaporation of 2 ml acetonitrile extract under a stream of nitrogen at 40 ^{o}C, thus obtaining final dilution factor of 2.5.

Method validation

Prior to AA analysis, the LC-MS/MS method was validated to ensure that the method performance characteristics meet the requirements laid down in EC Regulative (Commission Regulation, 2017). For this purpose, the method linearity was checked in the range 10-1000 µg/L, at six different concentrations. Furthermore, method accuracy (recovery) and precision (relative standard deviation) were studied preparing spiked samples of bread, French fries, and biscuits at two concentration levels of 200 and 400 µg/kg with 3 repetitions, during two different days. The main drawback for the validation study is the fact that there are no available blank samples for the recovery experiments. Therefore, samples with known amount of AA were spiked, and from the calculated total AA amount, the value obtained for the non-spiked samples was subtracted. Additionally, the Limit of Quantification (LOQ) was determined, to assess the method compliancy with the requirement laid down in EC Regulative (Commission Regulation, 2017).

Risk assessment in the population

The exposure data were estimated on basis on the contamination data obtained from the AA concentrations in the studied food commodities and the food consumption data published in the national statistical report for the households in North Macedonia for 2017 (State Statistical Office of Republic of North Macedonia, 2018). Chronic exposure was assessed for each food commodity group by multiplying the average daily consumption with the corresponding average occurrence level, summing up the respective intakes throughout the diet and finally dividing the results by assumed average body weight of 60 kg (EFSA, 2015). The average, standard deviation, median, as well the 25th, 75th, and 95th percentile was derived for each food commodity group.

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A reference point value for $BMDL_{10}$ was selected by EFSA (2015), 430 μg/kg_{bw}/day for peripheral neurotoxicity in rats and 170 μg/kg_{bw}/day for neoplastic effects in mice. Given these $BMDL_{10}$ values and the daily intake data derived from the contamination, the MOE data were calculated.

Statistical analysis

Statistical analysis was performed applying OriginPro 8 SR4 v8.0951 software package (OriginLab Corporation, Northampton, MA, USA). The calculated statistical parameters were average, Standard Deviation (SD), median, skewness, range (minimum-maximum), 25th percentile, 75th percentile, and 95th percentile. The statistical significance between the data series were analyzed at p<0.05. In order to estimate the average of AA contamination level, the left-censored data (results below LOQ) were treated by the substitution method, as recommended in EFSA (2015). Namely, two cases were considered for the treatment of results below the LOQ. Under the first case, occurrence values below LOQ were set at zero for the food groups with more than 60% left-censored results (lower bound scenario). For food commodity groups with less than 60% left-censored results, occurrence values below LOQ were set to half of the LOQ value (middle bound scenario).

Results

Prior to AA analysis, the LC-MS/MS method was validated to confirm the method performance requirements with the Commission Regulation. The method validation parameters were linearity, LOQ, accuracy, within-day, and between-day precision. The obtained validation data are presented in Table 2. Linearity was determined by five repetitions of six different concentrations: 10, 50, 100, 200, 500, and 1000 μg/L. LOQ was assessed at signal-to-noise level 10:1.

The determined levels associated with the SD for each commodity group, and the percent of AA-contaminated samples exceeding standard levels are presented in Table 3. Within the performed statistical analysis median, range and skewness were calculated. Significantly higher mean AA levels were found in French fries and potato crisps samples compared to all other food commodities (p<0.05). Additionally, the mean levels found in biscuits, wafers, cakes, and crisps were significantly higher than the levels found in bread, breakfast cereals, and pastry (p<0.05).

The exposure data presented in Table 4 were derived assuming that all population was exposed to AA through diet. Average value was 0.643±0.171 μg_{AA}/kg_{bw}/day, and the calculated 95th percentile (probable maximum exposure) was 1.110 μg_{AA}/kg_{bw}/day. On the basis of the data, one may conclude that the main contributor of AA in diet for overall population were bakery products i.e. bread contributing with 0.394±0.150 μg_{AA}/kg_{bw}/day (61.3% of the total intake) and with 95th percentile of 0.615 μg_{AA}/kg_{bw}/day. It was found that the average AA intake from bread was significantly differs from all other foods (p<0.05). The order of contribution to AA intake for all tested food commodity groups was as follows: bread>potato crisps>biscuits, wafers, cakes>crisps> French fries>breakfast cereals>pastry.

Calculations of average intake showed that the medium bound MOE value was 528 for neurotoxicity, with lower and upper bound values of 911 and 418. The lower, medium, and upper bound of the calculated MOE for 95th percentile were 416, 306, and 242, respectively. Regarding the neoplastic effects, given the average exposure data, the calculated MOE values were 360, 264, and 208 for lower, medium, and upper bound calculations. The 95th percentile values for MOE were 208, 153, and 121.

Table 1: Acrylamide and acrylamide-d_3 transitions and their corresponding cone voltage (V) and collision energies (eV)

Compounds	Transitions (*m/z*)	Cone voltage (V)	Collision energy (eV)
Acrylamide	72>42.4	26	8
	72>62.9	26	4
Acrylamide-d_3	75>58	20	6

Table 2: Validation parameters for the LC-MS/MS method for acrylamide

Validation parameters	Matrix					
	Potato crisps		Bread		Biscuits	
Spiking level (μg/kg)	100	300	100	200	100	200
Mean recovery (%; N=3)	87	88	91	85	86	95
Within-day precision (%; N=3)	4.6	6.5	4.8	4.2	5.7	4.4
Between-day precision (%; N=6)	6.0	8.3	7.2	6.5	6.8	5.3
Calibration equation (N=5); correlation	y=593.2x+11275; R^2=0.9958					
Limit of quantification	50 μg/kg (25 μg/kg for matrix bread and breakfast cereals)					

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Table 3: Acrylamide contents in foods sampled from North Macedonia

Sample type	Sample size	Mean±SD (µg/kg)	Median (µg/kg)	Range (µg/kg)	Skewness	Samples exceeding the indicative values* (%)
Bread	33	126.9±122.4	122.4	<25-195.9	-0.34	72.7
Biscuits, wafers, cakes	27	231.1±191.7	201.7	<50-666.8	+1.32	11.1
Crisps	25	252.0±176.4	231.7	<50-788.6	+1.35	12.0
Breakfast cereals	20	146.5±89.7	150.6	<50-305.6	-0.11	5.0
Pastry **	22	157.4±73.0	130.7	51.1-313.4	+0.87	0
French fries	14	494.5±127.1	541.0	214.3-657.4	-0.86	42.1
Potato crisps	19	390.6±192	360.6	168.4-861.0	1.03	5.3

*Increased values regarding the indicative levels according Commission Regulation (2017)
**Samples for which no indicative levels are laid down in Commission Regulation (2017)

Table 4: Assessment of acrylamide dietary intake (in $\mu g_{AA}/kg_{bw}$/day) for population from North Macedonia

Food category	Average	SD	Median	25th percentile	75th percentile	95th percentile
Bread	0.394	0.150	0.386	0.313	0.479	0.615
Biscuits, wafers, cakes	0.055	0.044	0.047	0.032	0.064	0.151
Crisps	0.042	0.029	0.039	0.027	0.048	0.089
Breakfast cereals	0.033	0.018	0.031	0.018	0.047	0.052
Pastries	0.013	0.006	0.011	0.009	0.017	0.026
French fries	0.041	0.011	0.045	0.034	0.049	0.054
Potato crisps	0.065	0.031	0.060	0.041	0.080	0.123
The overall intake	0.643	0.171	0.047	0.474	0.768	1.110

Discussion

This study is the first to report occurrence and estimate of the dietary exposure to AA of the population in North Macedonia. For the research purpose, we optimized and validated LC-MS/MS method for AA analysis in different food commodities. The determined mass spectrometric conditions were optimal for the instrument we used. One of the most important issues when analyzing low molecular weight compounds, as AA (M=71.08 g/mol), is minimizing the interference ions that increase the background signal from the mobile phase and the sample matrix. This is obtained using the MRM which is one of the MS/MS functions. The protonated AA molecular ion of 72 *m/z* and the fragment ions of 62.9 and 42.2 *m/z* obtained in positive electro spray ionization (ESI+) are structurally related to parent molecule. For the isotopically labeled AA obtained characteristic ion transition was 75>58 *m/z*. The cone voltage and collision energy for molecule fragmentation need to be determined for the instrument used in the current investigation in order to gain the most possible sensitivity. The criteria for confirmation of the AA identity in this method are in compliance with other LC-MS/MS studies, whereas the method applicability was tested on various food matrices. For instance, Mastovska and Lehotay (2006) developed a method for AA determination in potato chips, sweet potato chips, corn-based snacks, crackers, peanut butter, and chocolate. Also, Chen et al. (2012) developed an LC-MS/MS method for AA determination in variety of food samples such as crisps, chips, roasted nuts, hazelnut and peanut paste, traditional Chinese desserts, and cooked meals. The method developed by Kim et al. (2007) was tested for applicability on rice and bread samples, corn chips, and potato chips. Similarly, another analytical method for AA was previously developed, optimized, and validated only for crushed dry crackers (Roach et al., 2003).

The detector linearity for the optimized LC-MS/Ms method was checked by multiple injections of six calibration levels standards in the range 10-1000 ng/ml. The obtained average linearity was 0.9958 in the present research. The calibration solutions were in mobile phase, due to unavailability of true blank matrix which is necessary to perform the matrix calibration. On the other hand, the proposed QuEChERS procedure (Mastovska and Lehotay, 2006) was modified towards increasing laboratory sensitivity of the method, so, the sample amount for extraction was 2 g, and additional sorbent, bulk C18 phase, was used for dispersive solid-phase clean-up. This is especially important for removing of all fat residues from food commodities with high fat content.

Before analyzing the samples for AA content, it is mandatory to validate the used sample preparation proce-

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dure, to assess whether the method performances comply with the required criteria (Commission Regulation, 2017). These regulative requirements regarding the method performances are that repeatability less than 20%, recovery within the range 70-120%, LOQ≤50 μg/kg for benchmark levels higher than 125 μg/kg; for benchmark levels lower than 125 μg/kg, LOQ shall be less than two fifths of the benchmark level. All tested samples, except bread, had benchmark levels ≥125 μg/kg, thus the obtained LOQ value of 50 μg/kg was in line with the requirements. The desired LOQ for bread and breakfast cereals samples was obtained applying two-fold concentration of the final extract, thus the LOQ value was 25 μg/kg. Considering the validation parameters presented in Table 2, it may be seen that the applied method has fulfilled the Regulative (Commission Regulation, 2017) requirements. When comparing the obtained LOQ values for this method and previously published data (Chen et al., 2012; Roach et al., 2003), it could be stated that the LOQ values within this study are 5-10 times higher. The reason for this were probably high mobile phase and matrix interferences that occur when analyzing low molecular fragments, as it was for AA (Table 1). Additionally, the applied mass spectrometric detector was able to detect mass fragments within the range of 40 to 2000 *m/z*, meaning that the AA target fragments (42.4 *m/z* and 62.9 *m/z*) are close to the lowest instrument mass detection range.

The AA levels, and accordingly the contribution to the AA intake, among different food groups and among the samples within the same food group vary significantly depending on the quality of raw materials, food formulation, manufacturing conditions, and mitigation measures taken. The contribution of the individual food groups and food commodities to the total intake is not only defined by their AA levels, but also by the amount consumed. The typical example for this is the obtained data for bread contribution to the total AA intake. Namely, even though the average contamination level for our bread samples was 126.9±122.4 μg/kg, due to high consumption, bread was the main contributor for AA in the diet. Hence, for different types of bread, the average intake was 0.394±0.150 $\mu g_{AA}/kg_{bw}$/day and the 95th percentile 0.615 $\mu g_{AA}/kg_{bw}$/day. The second biggest one contributor were potato crisps with an intake of 0.065 $\mu g_{AA}/kg_{bw}$/day and 0.123 $\mu g_{AA}/kg_{bw}$/day for the 95th percentile. It has to be notified that the food consumption data were derived from the official statistics for food consumption in 2017 for the population from North Macedonia (State Statistical Office of Republic of North Macedonia, 2018) and may not be realistic for different population age categories. The intake estimation in this research was performed on general approximation for average body weight of 60 kg; therefore, the consumption difference for various age groups was not taken into consideration that is limitation of our study. The main reason for this was the lack of data for consumption habits for different age groups. The WHO (2002) reported that in general, AA intake in children is two to three fold higher than in adults, expressed on body weight basis. In addition, children and adolescents prone to consume AA reach food.

Previous data revealed that in Southern Poland for population age from 6 to 12 years, the overall intake of AA was 1.51 $\mu g_{AA}/kg_{bw}$/day, and for population age from 42 to 60 years, the overall intake was 0.67 $\mu g_{AA}/kg_{bw}$/day (Zając et al., 2013), which is comparable to the intake data from our study (0.643 $\mu g_{AA}/kg_{bw}$/day). The current investigation for AA assessment revealed intake that was similar with Hungarian data and about two times higher than the national surveys conducted in Belgium, France, as well as Norway; but 60-70% higher than the Danish exposure (EFSA, 2011). The higher intake showed in our research is probably resulted from nonexistence of strict monitoring programs for AA in food, and further more lack of regulation for mitigation measures that should be undertaken by food operators. However, the intake of 0.643 $\mu g_{AA}/kg_{bw}$/day determined in this work, lays within the range 0.5-1.9 $\mu g_{AA}/kg_{bw}$/day, that was determined in European Union countries during the survey conducted in the period 2010-2014 (EFSA, 2015).

According to the data collected from The Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2011), the intake assessment varied from 0.3 to 2.0 $\mu g_{AA}/kg_{bw}$/day among 17 countries worldwide. The differences in assessment surveys are very probably due to the differences in population age, methods used, time of study conduction, and products taken under consideration. No less important, it has to be emphasized that the differences in determined intakes arise from the different dietary and cooking habits among the populations in each country. Acrylamide is formed during the food heating because of Maillard reaction. During the heating process independently whether the frying of baking is performed, the free amino acid asparagine is decarboxylated and deaminated by the intermediate reaction with reducing sugars or other carbonyl compounds, which results in AA formation (Anese et al., 2010; JECFA, 2011). Additionally, other mechanisms for AA formations have been identified, including the pyrolysis of gluten or initial enzymatic decarboxylation of asparagine in raw potatoes. Therefore, the Commission Regulation (2017) has set obligatory mitigation measures for reduction of AA forming during the heating processes. The foreseen mitigation measures include control of raw materials for asparagine and reducing sugars, control of heating temperature for each type of food, as well as use of some food additives for lowering pH value in dough at bakery products (Keramat et al., 2011; Vinci et al., 2012).

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The MOE is the ratio between a particular point on the dose response curve leading to tumors in experimental animals and the exposure (EFSA, 2011). The size of the MOE value is an indicator about the possible extent of the risk, i.e. the higher the MOE, the lower the risk of exposure to the respective compound. According to JECFA's report (JECFA, 2011), MOE values between 45 and 310 may implicate health concern. In our study the MOE medium-bound value for the average intake of 0.643 $\mu g_{AA}/kg_{bw}$/day was 528 and 264 for peripheral neurotoxicity and neoplastic effects, respectively. The determined upper-bound MOE for the calculated 95th percentile was 242 for neurotoxicity and 121 for neoplastic effects. Usually, for non-genotoxic compound a MOE value of 100 is considered as the lowest value to conclude that there is no health risk (EFSA, 2015). This MOE takes into account the uncertainties and variability regarding both kinetic and dynamic differences between experimental animals and humans.

Considering the medium bound values for high exposure data in the current study (the calculated 95th percentile), the respective MOE data for peripheral neurotoxicity was 388 and for neoplastic effect was 153. Having on mind the fact that the intake was calculated on average body weight value of 60 kg, it is obvious that for younger population of infants and toddlers with lower body weight, the risk for occurrence of neoplastic effects increases due to AA intake, with upper-bound MOE value of 121 for the calculated 95th percentile value.

This study determined bakery products (i.e. bread) as the main source of AA contamination. According to the data reported for bread consumption in the State Statistical Office of Republic of North Macedonia (2018), bread in North Macedonia is consumed in amount around 70 kg per inhabitant yearly. The other studies for national AA exposure reported yearly consumption per inhabitant of 60 kg in Poland (Zając et al., 2013) and 83 kg in Germany (Hilbig et al., 2004). In all these studies, the AA intake from bakery products was predominant. On the other hand, the investigations conducted in Belgium and Nederland identified the crisps as a main source of AA intake, while in France and USA studies, French fries and potato crisps, respectively, were predominant in AA intake (Zając et al., 2013).

The results from the present study for AA content in food and furthermore the population intake emphasize the importance of AA monitoring as reflected in Commission Regulation (Commission Regulation, 2013) and undertaking the necessary mitigation strategies and measures by food producers as laid down in EC Regulative (Commission Regulation, 2017). For estimation of more accurate intake and exposure data, it is necessary to conduct food frequency questionnaire in North Macedonia which will cover different population age groups. In this way, the obtained exposure data would give a much clearer picture of the real risk from consumption of AA-rich foods, especially for the adolescent population.

Conclusion

The current study presented the method capability for determination of AA levels in different food commodities. The contamination data indicated that the indicative AA levels were mostly exceeded in tested bread samples. The risk assessment analysis revealed increased concern for human health regarding the neoplastic effects, especially for infants, toddlers, and adolescents. This is the first study related to AA presence in different food commodities in North Macedonia, and implied that monitoring programs and mitigation strategies must be implemented. For more accurate intake and exposure data, it is necessary to conduct food frequency questionnaire, which will cover different consumer age groups.

Author contributions

E.D-S. and Z.H-M. designed the study; E.D-S. wrote the manuscript; A.A., D.K., and G.I. conducted the experiments; R.U. performed the validation experiments; E.D.-S., B.S.-D., and G.S. analyzed the data; B.S-D. and D.J. revised the manuscript. All authors revised and approved the final manuscript.

Conflicts of interest

The authors report no potential conflict of interest.

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