ACRYLAMIDE CONTENT IN SELECTED FOOD PRODUCTS COLLECTED FROM MONTENEGRIN MARKET AND HEALTH RISK ASSESSMENT

Dijana Đurović[1, 2], Miljan Bigović[3], Ljubica Ivanović[1], Maja Blagojević[4], Amil Orahovac[2]
[1]Institute of Public Health of Montenegro, Podgorica, Montenegro
[2]Faculty for Food Technology, Food Safety and Ecology, University Donja Gorica, Podgorica, Montenegro
[3]Faculty of Science and Mathematics, University of Montenegro, Podgorica, Montenegro
[4]Biotechnical Faculty, University of Montenegro, Podgorica, Montenegro

SUMMARY

Objectives: This study aimed to investigate the acrylamide exposure of different children and adult population groups (10–14 years, 15–17 years, 18–24 years, 25–44 years, 45–64 years, and 65–74 years) resulting from the consumption of potato chips and wheat-based bread from Montenegrin market and to evaluate it in terms of health risk.

Methods: The acrylamide content was monitored in 51 samples of bread and 20 samples of chips. The carcinogenic health risk in different population groups was assessed through the incremental lifetime cancer risk (CR) and total cancer risk (TCR).

Results: The average acrylamide content in potato chips and bread was calculated to be 238 μg/kg and 30 μg/kg, respectively. Acrylamide content in a tested sample met the criteria prescribed by Commission Regulation (EU) 2017/2158 in 98% of the tested samples of chips and 85% of bread samples. The carcinogenic health risk of acrylamide exposure for the investigated population groups is of concern. The values of CR for all the investigated groups were in the range of $10^{-6} < CR < 10^{-4}$ and the values of TCR were 10^{-5} order of magnitude, indicating a potential cancer risk.

Conclusion: The youngest population (10–14 years) was exposed to the highest cancer risk through the consumption of both, chips and bread. For the population of 10–14 years, 15–17 years, 25–44 years, 45–64 years, and 65–74 years, a higher risk of cancer was found due to the consumption of bread compared to the consumption of chips. Only the population aged 18–24 years was faced with a higher risk of cancer due to the consumption of chips compared to the consumption of bread.

Key words: acrylamide, potato chips, wheat bread, daily intake, health risk

Address for correspondence: D. Đurović, Institute of Public Health of Montenegro, bb John Jackson Street, 81000 Podgorica, Montenegro. E-mail: dijana.djurovic@ijzcg.me

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INTRODUCTION

Acrylamide (prop-2-enamide) is a solid organic compound, well soluble in water, methanol and ethanol. It is mainly used in the chemical industry for polyacrylamide synthesis which is further widely used in the industry of textile, paper and cosmetics, as insulating material, in water tanks, reservoirs, wastewater treatment, and construction (1). The presence of acrylamide in food (chips, bread, cereals, coffee, French fries) that was subjected to thermal treatment at temperatures above 120 ºC was discovered in 2002 (2). Acrylamide is mainly formed in foods rich in carbohydrates and asparagine with low protein content and in low moisture conditions (3, 4). These are mainly food products based on cereals, potatoes and coffee. The content of acrylamide in food depends on the type of food and its thermal treatment (temperature and time) (5). Generally, the acrylamide content in food increases with the rise in temperature and time of food thermal treatment (6).

Studies conducted on rodents have shown that acrylamide exerts its mutagenicity via metabolism by CYP2E1 to glycidamide (GA), induce gene mutations by a pathway involving the generation of reactive oxygen species (ROS) and oxidative DNA damage (7, 8).

Neurotoxicity is the only toxic effect of acrylamide that has been proven on humans and animals, while genotoxicity and carcinogenicity have been proven on animals (9). Acrylamide was evaluated by the International Agency for Research on Cancer (IARC) and classified as a probable carcinogen of class 2A (10). Considering the harmful effect of acrylamide on human health, the European Commission published Regulation (EU) 2017/2158 establishing mitigation measures and benchmark levels to reduce acrylamide in foods (11) and Regulation (EU) 2020/2184 on the quality of water intended for human consumption (12).

Since bread and potato chips are among the food items that contain the highest levels of acrylamide and are highly consumed food items in Montenegro, this study aimed to assess the content of acrylamide in these food products and the health risk in different population groups in Montenegro.

MATERIALS AND METHODS

Sample Preparation

A total of 71 samples (51 samples of bread and 20 samples of potato chips) were purchased from five different local markets in Podgorica, Montenegro. The potato chips samples analysed for acrylamide content were purchased in 100 g packages, while for bread analysis, a total of 51 samples of bread loaves were sampled. The entire packages of potato chips (100 g) and the whole loaves of bread (500 g) were homogenized separately in a food processor before sampling. The homogenized food samples were sampled directly, and the amount of 1 g of potato chips and 5 g of bread samples were weighed separately into 50 mL centrifuge tubes. To defat the samples, 5 ml of n-hexane was added, and the tube was vortexed for 1 minute. The applied analytical method for acrylamide determination is a slightly modified protocol presented by Stefanović et al. (13), using QuEChERS for extraction and purification according to EN 15662 protocol (14). After the samples were weighed, the test portions were diluted with 10 mL of deionized water and vortexed, 10 ml of acetonitrile was added, and the mixture was shaken for 10 minutes at an ultrasonic bath. After that, the mixture of salt and buffer (4 g $MgSO_4$, 1 g NaCl, 1 g tri-Sodium citrate dihydrate, 0.5 g disodium hydrogen citrate) was added to the tube. After shaking for 1 minute, the sample was centrifuged at 4,500 rpm for 5 minutes. In samples where hexane was added, the hexane layer was removed from the tube using a pipette. The sample purification was then continued by transferring 6 mL of the acetonitrile layer into a 6 ml centrifuge tube containing 150 mg of PSA and 900 mg of anhydrous $MgSO_4$. The centrifuge tube was closed up, shaken vigorously for 1 minute, and then centrifuged for 5 minutes at 4,500 rpm. The 0.7 ml of clean supernatant was filtered through a 0.2 µm syringe filter, mixed with 7 µl 5% formic acid and injected into the LC-MS system.

Quality assurance and quality control for the applied method were achieved using the following certified reference materials: FAPAS 3080 potato crisps and FAPAS T30140QC French fries.

Method

Acrylamide content in the food samples was determined using an Agilent 1260 Infinity LC system coupled with an Agilent 6465B triple quadrupole LC-MS system. Instrument control, data acquisition, qualitative and quantitative data analysis, and reporting were done using Agilent MassHunter workstation software. The separation was carried out on an Agilent Extend-C18 column 3.0 × 150 mm, 3.5 µm at a flow rate of 0.2 mL/min and temperature maintained at 25 °C. The mobile phase consisted of water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B). The gradient employed was the following: 0–5 min, 90–10% B; 6–6 min, 10–90% B; 6–7 min, maintained to 90% B. The injection volume was 5 µL. Mass spectrometric determination of acrylamide was performed in an electrospray (ESI) positive mode. The optimized MS instrument parameters were the drying gas temperature (N2) of 300 °C with a flow rate of 11 L min^{-1}, nebulizer pressure of 15 psi, a capillary voltage of 4.5 kV and fragmentor voltage of 85 V for acrylamide. The detector was operating in a multiple reaction monitoring (MRM) mode. Two product ions of acrylamide were monitored (m/z 72 > 55 and 72 > 44), the collision energy of 5 and 8 V for used transitions, respectively. Dwell time was set to 100 ms.

Health Risk Assessment

Daily intake of acrylamide was estimated for different population groups of children (10–14 years and 15–17 years) and adults (18–24 years, 25–44 years, 45–64 years, and 65–74 years) according to the formula

$$CDI = \frac{C_i \cdot IR_i \cdot ED_i \cdot EF_i}{BW \cdot AT}$$

where CDI (µg/kg/day) is the daily intake of i-food (bread or chips), C_i is the concentration of acrylamide in i-food (µg/kg), IR_i is the intake rate of i-food (g/day) for each population group, ED_i is exposure duration (year), EF_i is exposure frequency (365 day/year), BW as average body weight (kg), and AT is the average exposure time ($AT = EF_i \cdot ED_i$). The parameters used for CDI calculation are summarized in Table 1.

Table 1. *Parameters used for health risk assessment*

Parameter		Population					
		Children (10–14 years)	Children (15–17 years)	Adults (18–24 years)	Adults (25–44 years)	Adults (45–64 years)	Adults (65–74 years)
IR_i (g/day)	Chips	5.28	4.59	5.96	2.51	1.03	0.78
	Bread	88.63	74.89	81.07	89.95	102.30	97.08
ED_i (year)		6	6	70	70	70	70
EF_i (day/year)		365	365	365	365	365	365
BW (kg)		51.74	69.34	75.52	78.96	82.42	81.22
AT (day)		2190	2190	25550	25550	25550	25550
RfD (mg/kg/day)			0.002	0.002	0.002	0.002	0.002
CSF $(mg/kg/day)^{-1}$		0.5	0.5	0.5	0.5	0.5	0.5

IR_i – intake rate of i-food; ED_i – exposure duration; EF_i – exposure frequency; BW – body weight; AT – average exposure time; RfD – reference dose; CSF – cancer slope factor
Source: Stefanović et al. (13), EN 15662/2018 (14), USEPA (15)

Carcinogenic health risk was assessed using the incremental lifetime cancer risk (CR) and total cancer risk (TCR) calculated according to the equation 2 and equation 3, respectively (15). The value of CR indicates the cancer risk that a human may be exposed to for their lifetime due to a carcinogenic or potentially carcinogenic substance.

Equation 2:

$$CR = CDI_i \cdot CSF$$

Equation 3:

$$TCR = \sum CR_i$$

were CSF refers to the cancer oral slope factor which for acrylamide is 0.5 mg/kg/day for each population group (Table 1). Values of $CR \leq 10^{-6}$ indicate an acceptable or insignificant cancer risk, values of $CR \geq 10^{-4}$ indicate a serious cancer risk while values of CR values in the range of $10^{-6} < CR < 10^{-4}$ indicate a potential risk of cancer.

Table 2. *Descriptive statistics of acrylamide content (μg/kg) in selected food sample*

Patrameter	Chips (n = 51)	Bread (n = 20)
Average	238	30
Minimum	49	15
Maximum	1,089	108
Median	190	22
Standard deviation	31	24

RESULTS

Acrylamide Content in Selected Food Products

Acrylamide content in the samples of potato chips and bread is given in Figure 1 and 2, and descriptive statistics in Table 2. The

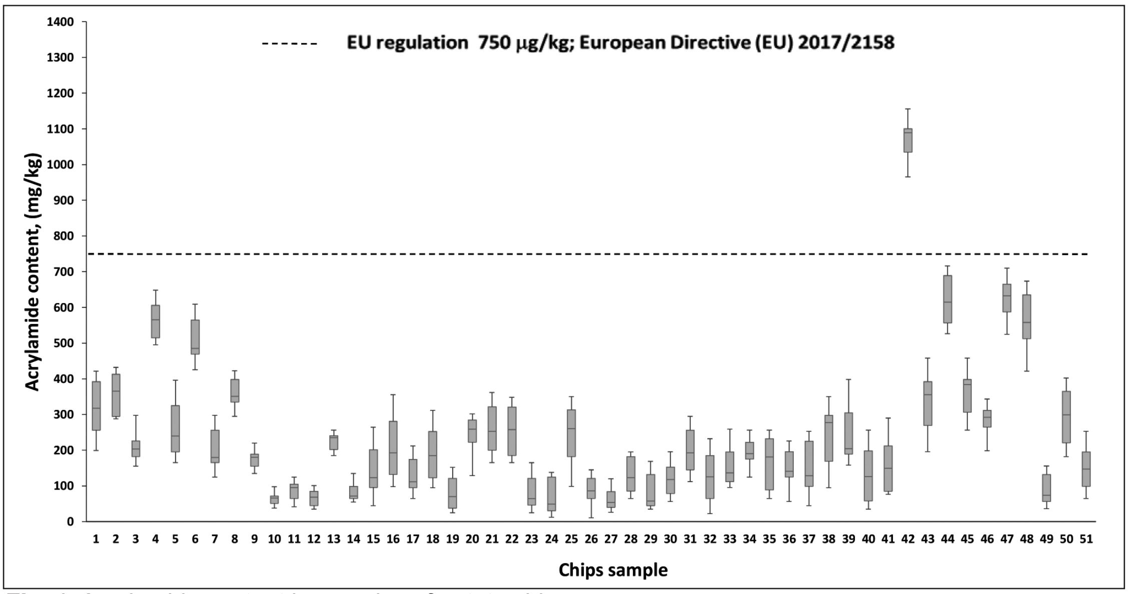

Fig. 1. *Acrylamide content in samples of potato chips.*

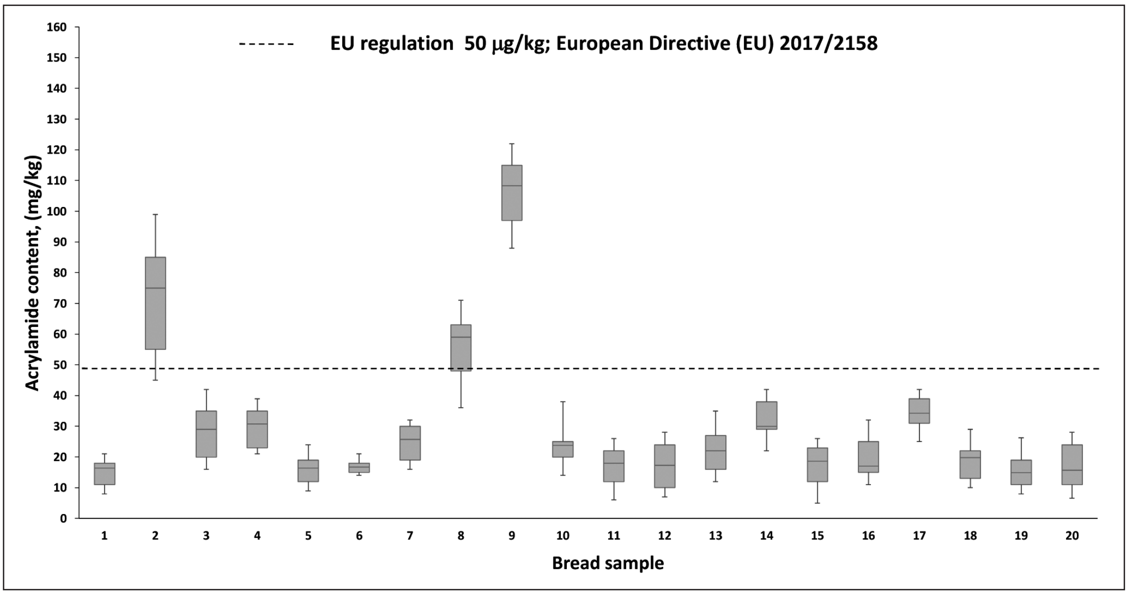

Fig. 2. *Acrylamide content in bread samples.*

results presented in Figure 1 indicate that acrylamide content in the samples of potato chips was in the range of 49 μg/kg to 1,089 μg/kg while in the samples of bread, this content ranged between 15 μg/kg and 108 μg/kg. The average values of acrylamide content in potato chips and bread were 238 μg/kg and 30 μg/kg, respectively (Table 2). The average content of acrylamide in both selected food samples was below the prescribed limits proposed by Commission Regulation (EU) 2017/2158: 750 μg/kg for potato chips and 50 μg/kg for wheat-based bread (11).

The results presented in Figure 1 indicate that acrylamide content in 2% of the investigated samples of potato chips exceeded the values (750 μg/kg) proposed by EU Regulation (11) while in the case of the bread sample (Fig. 2), acrylamide content exceeded the prescribed value (50 μg/kg) in 15% samples. The values of acrylamide content in the selected food samples were compared with the values of acrylamide in potato chips and wheat-based bread obtained by different studies.

Daily Intake of Acrylamide

The average daily intake of acrylamide through the consumption of chips and bread in different age groups of the Montenegrin population is given in Table 3. The daily intake of acrylamide through the chips consumption in the Montenegrin population follows a descending order: $CDI_{(10–14 years)} > CDI_{(18–24 years)} > CDI_{(15–17 years)} > CDI_{(25–44 years)} > CDI_{(45–64 years)} > CDI_{(65–74 years)}$ while for bread consumption the values of daily intake follow the following descending order: $CDI_{(10–14 years)} > CDI_{(45–64years)} > CDI_{(65–74 years)} > CDI_{i(25–44 years)} > CDI_{(15–17 years)} > CDI_{(18–24 years)}$. As shown, the highest acrylamide daily intake (0.024 μg/kg/day) through chips consumption was attributed to the youngest population aged 10–14 years followed by the daily intake of the populations aged 18–24 years (0.019 μg/kg/day) and 15–17 years (0.016 μg/kg/day). The lowest values of acrylamide daily intake through chips consumption were calculated to be 0.008 μg/kg/day, 0.003 μg/kg/day and 0.002 μg/kg/day for adult groups 25–44 years, 45–64 years and 65–74 years, respectively.

As for the consumption of bread, the highest (0.051μg/kg/day) and lowest (0.002 μg/kg/day) estimated acrylamide daily intake was attributed to the populations aged 10–14 years and 18–24 years, respectively. Very similar values of bread daily intake of 0.034 μg/kg/day, 0.036 μg/kg/day and 0.037 μg/kg/day were obtained for the population of 45–64 years, 65–74 years and 25–44 years old, respectively.

Health Risk Assessment

The results of carcinogenic health risk assessment are given in Table 4 and Figure 3. The results have shown that all the populations were faced with carcinogenic health risk since calculated CR values were higher than $1 \cdot 10^{-6}$ (Table 4). The obtained average values of cancer risk for chips consumption in the investigated populations can be arranged in a descending order as follows: $CR_{(10–14 years)} > CR_{(18–24 years)} > CR_{(15–17 years)} > CR_{(25–44 years)} > CR_{(45–64 years)} > CR_{(65–74 years)}$. The youngest population (10–14 years) faced the highest carcinogenic risk of $1.21 \cdot 10^{-5}$. The obtained CR values for other population groups were: $9.41 \cdot 10^{-6}$, $7.89 \cdot 10^{-6}$, $3.79 \cdot 10^{-6}$, $1.49 \cdot 10^{-6}$, and $1.14 \cdot 10^{-6}$ for the population of 18–24 years, 15–47 years, 25–44 years, 45–64, and 65–74 years, respectively indicating the potential carcinogenic risk of chips consumption.

As for consumption of bread, the obtained values of cancer risk were in the range of $1.18 \cdot 10^{-6}$ and $2.57 \cdot 10^{-5}$ and can be arranged in descending order as follows: $CR_{(10–14 years)} > CR_{(45–64 years)} > CR_{(65–74 years)} > CR_{i(25–44 years)} > CR_{(15–17 years)} > CR_{(18–24 years)}$. Again, the youngest population (10–14 years) faced the highest carcinogenic risk ($CR = 2.57 \cdot 10^{-5}$) followed by the population of 45–64 years ($CR = 1.86 \cdot 10^{-5}$), 65–74 years ($CR = 1.79 \cdot 10^{-5}$), 25–44 years ($CR = 1.71 \cdot 10^{-5}$), and 15–17 years ($CR = 1.62 \cdot 10^{-5}$). The lowest carcinogenic risk ($CR = 1.18 \cdot 10^{-5}$) of bread consumption was obtained for the population aged 18–24 years. Since the values of carcinogenic risk of the consumption of both potato chips and bread were in the range of 10^{-6} and 10^{-4}, all the investigated populations faced the potential carcinogenic risk (15). To assess the health risk due to the combined effect of chips and bread consumption, the total cancer risk (TCR) was calculated and the values obtained were 10^{-5} orders of magnitude (Table 4) for all the investigated populations, indicating also potential carcinogenic health risk. The highest total cancer risk was obtained for the population of 10–14 years and the population of 18–24 years faced the lowest cancer risk. The values of total cancer risk for the investigated populations were in the following order: $TCR_{(10–14 years)} > TCR_{(15–17 years)} > TC_{(25–44 years)} > TCR_{(45–64 years)} > TCR_{(65–74 years)} > TCR_{(18–24 years)}$.

Table 3. *Daily intake (μg/kg/day) of selected foods containing acrylamide in the Montenegrin population*

Food sample	Parameter	Population					
		Children (10–14 years)	Children (15–17 years)	Adults (18–24 years)	Adults (25–44 years)	Adults (45–64 years)	Adults (65–74 years)
Chips	Average	0.024	0.016	0.019	0.008	0.003	0.002
	Minimum	0.005	0.003	0.004	0.002	0.001	0.001
	Maximum	0.111	0.072	0.086	0.035	0.014	0.010
	SD	0.019	0.013	0.015	0.006	0.002	0.002
Bread	Average	0.051	0.032	0.002	0.034	0.037	0.036
	Minimum	0.026	0.016	0.001	0.017	0.019	0.018
	Maximum	0.186	0.117	0.008	0.123	0.134	0.129
	SD	0.041	0.026	0.002	0.027	0.029	0.028

SD – standard deviation

Table 4. Carcinogenic risk of dietary acrylamide exposure of different population groups

Food sample	Parameter	Cancer risk					
		Population					
		Children (10–14 years)	Children (15–17 years)	Adults (18–24 years)	Adults (25–44 years)	Adults (45–64 years)	Adults (65–74 years)
Chips	Average	1.21E-05	7.89E-06	9.41E-06	3.79E-06	1.49E-06	1.14E-06
	Minimum	2.50E-06	1.63E-06	1.95E-06	7.83E-07	3.08E-07	2.37E-07
	Maximum	5.53E-05	3.61E-05	4.30E-05	1.73E-05	6.81E-06	5.23E-06
	SD	9.72E-06	6.33E-06	7.55E-06	3.04E-06	1.20E-06	9.19E-07
Bread	Average	2.57E-05	1.62E-05	1.18E-06	1.71E-05	1.86E-05	1.79E-05
	Minimum	1.28E-05	8.07E-06	5.86E-07	8.51E-06	9.27E-06	8.93E-06
	Maximum	9.28E-05	5.85E-05	4.24E-06	6.17E-05	6.72E-05	6.47E-05
	SD	2.04E-05	1.28E-05	9.31E-07	1.35E-05	1.47E-05	1.42E-05
		Total cancer risk					
		Population					
		Children (10–14 years)	Children (15–17 years)	Children (10–14 years)	Children (15–17 years)	Children (10–14 years)	Children (15–17 years)
		3.78E-05	2.41E-05	1.06E-05	2.09E-05	2.01E-05	1.91E-05

SD – standard deviation

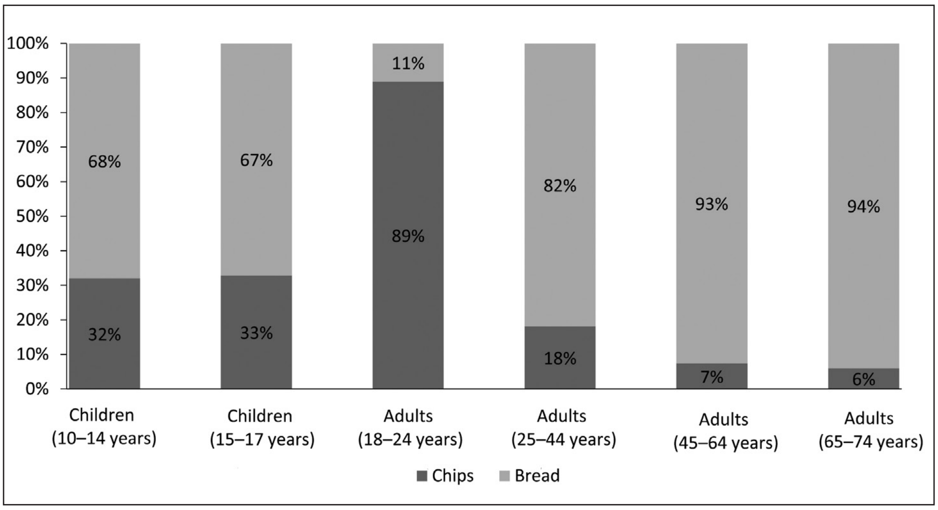

Fig. 3. *Contribution of chips and bread consumption to total cancer risk for different population groups.*

The contribution of different food products to carcinogenic health risk for the investigated population groups is given in Figure 3. It is evident that only for the population of 18–24 years chips consumption contributes (89%) to the total cancer risk more than bread consumption (11%), while for the other population groups the higher contribution of bread consumption was observed. For younger population groups, 10–14 years and 15–17 years, bread consumption contributes to the total cancer risk with 68% and 67%, respectively, while chips contribute with 32% and 33%, respectively. For the population group of 25–44 years, contributions of bread and chips consumption were 82% and 18%, respectively. Similar contributions of the selected food products were obtained for the populations of 45–64 years and 65–74 years: 93% and 94% for bread consumption, and 7% and 6% for chips consumption, respectively (Fig. 3).

DISCUSSION

The average value of acrylamide content in potato chips obtained in this study was lower in comparison to the results reported in chips samples in the Czech Republic (982 µg/kg) (5), Spain (1,484 µg/kg) (16), Brazil (591 µg/kg) (17), India (1,534 µg/kg) (18), Canada (2,453 µg/kg) (19), and in a study conducted by the European Agency for Food Safety (EFSA) (758 µg/kg) (20). However, the acrylamide content in this study was higher in comparison to potato chips from the Austrian market (169 µg/kg) (21), and the Romanian market (134 µg/kg) (22). On the other hand, the acrylamide content in bread in this study was lower than in bread samples found in Poland (59 µg/kg) (23), Germany (916 µg/kg) (24), Belgium (118 µg/kg) (25), and Slovenia (135 µg/kg) (26).

Daily intake of acrylamide calculated for children (10–14 years) for both selected food products is typically higher than for older population groups in part due to their higher consumption of chips and bread in comparison to adults (27).

The differences between the population groups in terms of food products that contribute most significantly to the total cancerogenic risk of acrylamide exposure can be primarily attributed to differences in consumption habits (28).

CONCLUSIONS

This study revealed the acrylamide content in potato chips and wheat-based bread collected from Montenegrin market. The acrylamide content in chips samples ranged from 49 µg/kg to 1,089 µg/kg and in bread samples from 15 µg/kg to 108 µg/kg. The vast majority of the chip samples (98%) complied with the Commission Regulation 2017/2158 while only 85% of bread samples were in line with the Regulation. The highest daily intake of acrylamide through chips and bread consumption was obtained for the population of 10–14 years. The results have shown that all the investigated population groups are exposed to carcinogenic health risk of acrylamide exposure through the consumption of both products. The youngest population (10–14 years) faces the highest carcinogenic risk due to the consumption of both, chips and bread. For the population aged 18–24, chips consumption contributes more to the total cancer risk of acrylamide exposure in comparison to the consumption of bread. For all the other investigated populations (15–17 years, 25–44 years, 45–64 years, and 65–74 years) the consumption of bread has a greater contribution to the total cancer risk of acrylamide exposure than the chips consumption.

Conflicts of Interest

None declared

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