DETERMINATION OF ACRYLAMIDE LEVELS IN SELECTED FOODS IN LATVIA AND ASSESSMENT OF THE POPULATION INTAKE

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Abstract

The aim of this study was to investigate acrylamide levels in foods obtained from the Latvian market. Eight sample groups including traditional Latvian food products (rye bread, rye bread enriched with seeds, dried fruits or vegetables, fine rye bread (sweet-and-sour bread), wheat bread, potato chips, coffee, pastry products, dried bread products and gingerbread) were analyzed for their acrylamide content. A total of 435 samples were analyzed for acrylamide concentration using the ultra high performance liquid chromatography with tandem mass spectrometric detector and QuEChERS sample preparation methodology. An appropriate average recovery (106%), within-laboratory repeatability (3–10%) and limit of quantification (10 µg kg^{-1}) was specific to the applied UPLC-MS/MS methodology. Results revealed that the acrylamide content of processed foods shows a great variation between different food groups as well as between brands and within brands. Highest concentration of acrylamide was revealed in potato chips and chicory coffee with average concentration 564 µg kg^{-1} and 2790 µg kg^{-1} respectively. No single bread sample has exceeded the European Commission Recommendation 2013/647/EU although the addition of seeds, fruits or vegetables clearly had an increasing effect on acrylamide concentration in bread. The dietary exposure assessment was performed, using analytical results on acrylamide levels in certain food groups and the relevant food consumption data. The calculated data indicates high dietary intake of acrylamide by certain consumers that may potentially cause adverse health effects. Therefore, adequate efforts should be made to diminish acrylamide levels in processed foods in order to reduce the dietary risk to the human health for Latvian population.

Keywords: acrylamide, Latvian food, population intake.

Introduction

Acrylamide, a thermal process-induced contaminant in food, has attracted worldwide researchers to study the mechanism of its formation in foods, the risks associated for consumers and possible strategies to lower acrylamide levels in foodstuffs (Lui et al., 2013).

The International Agency for Research on Cancer, taking into consideration an option of industrial exposure to this compound and its intake from drinking water and tobacco smoke, classified acrylamide already in 1994 as a compound „probably carcinogenic for humans" (Group 2A) (IARC, 1994). Experimental studies in animals have shown genotoxic and carcinogenic effects of acrylamide. The genotoxicity of acrylamide could be partly mediated by its main metabolite, glycidamide (Gamboa da Costa et al., 2003; Baum et al., 2005) which is mutagenic (Blasiak et al., 2004; Ghanayem et al., 2005).

Since April 2002, when the Swedish National Food Administration published for the first data set about high content of acrylamide in food, several studies in different countries were performed dedicated to the assessment of acrylamide content in foods. The presence of acrylamide in different foodstuffs and its toxic impact, stirred up interest on a global scale in a possible risk to human health. Consequently, many countries have started to estimate acrylamide dietary intake (Mojska et al., 2012).

Currently it is well known that acrylamide is mainly formed as a result of reaction between amino acids and reducing sugars (particularly glucose and fructose) as a part of Maillard reaction (Mottram et al., 2002; Stadler, Scholz, 2004). Studies also have shown that acrylamide formation is affected by several processing parameters, such as heating temperatures, duration of heating, reducing sugar content, addition of components that bind water, and surface to volume ration (Boon et al., 2005)

The main source of acrylamide in the diet are primarily products made of potato such as French fries and potato crisps, and also cereal products such as bread, breakfast cereals, cookies and biscuits. Acrylamide is also formed during the coffee roasting process (Mojska et al., 2012)

Due to multiple sources of dietary exposure to acrylamide, a consultation was held in June 2002 by the Food and Agriculture Organization/World Health Organization (FAO/WHO) to discuss possible health risks due to acrylamide (WHO, 2002). Based on the expert committee evaluation, 1 µg kg^{-1} body weight (bw) day^{-1} is considered as an average exposure to acrylamide while 4 µg kg^{-1} bw day^{-1} is considered as high exposure to acrylamide (JECFA, 2006). The mean dietary exposure range to acrylamide is 0.2–1.0 µg kg^{-1} bw day^{-1} for the general adult population while 95th percentile range is 0.6–1.8 µg kg^{-1} bw day^{-1} (JECFA, 2011)

Currently, acrylamide is monitored in the European Union according to the European Commission Recommendation (2010/307/EU). Indicative acrylamide values based on the EFSA (European Food Safety Authority) monitoring data from 2011 to 2012 were established within the European Commission recommendation released on November 8, 2013 ("On investigations into the levels of acrylamide in food"). The indicative values are not safety thresholds, but, if exceeded, subsequent investigation of the reasons should be conducted.

To sum up significant variation of acrylamide content within the Latvian food products was obtained mainly as a result of the variable critical process parameters (critical temperature threshold, processing time, etc.) which are characteristic for each type of food, manufacturer, product brand, even the batch. The content of acrylamide in traditional Latvian origin products (rye bread and rye bread with addition of seeds and fruit) is in line with the EU recommendation 2010/307/EU. The data presented here for other Latvian foods are in the same range as published in other countries.

The aim of this study was to investigate acrylamide levels in foods obtained from the Latvian market and to assess the average dietary acrylamide exposure of the Latvian population.

Materials and Methods

Food sampling

Given that acrylamide is formed in heat-treated potato and cereal products such e.g. bread, breakfast cereals, cookies and biscuits and during the coffee roasting process, the food products produced in Latvia were selected for testing purposes that might be a prospective source of dietary acrylamide intake for the Latvian population.

Eight product groups were selected for the study:

1) rye bread, rye bread enriched with seeds, dried fruits or vegetables;
2) sweet-and-sour bread;
3) wheat bread;
4) potato chips;
5) coffee, including instant;
6) confectionery (cookies, crackers);
7) gingerbread;
8) bread products (bagels, crackers, etc.).

A total of 435 samples obtained from all Latvian regions were analyzed for the acrylamide content.

Chemicals

Acrylamide (99%) and acrylamide-d_3 standards were obtained from Sigma (St. Louis, MO, USA). Stock and working standard solutions of acrylamide and acrylamide-d_3 were prepared in acetonitrile. Methanol, hexane and acetonitrile were a gradient grade for HPLC and formic acid (98%) from Sigma (St. Louis, MO, USA). Deionized water was prepared by a Milli-Q (Millipore, Billerica, MA, USA) water purification system. Sodium chloride and anhydrous magnesium sulfate were purchased from Scharlau (Barcelona, Spain). Primary secondary amine (PSA) sorbent was purchased as a bulk sorbents from UCT (Brockville, ON, Canada).

Analysis by LC-MS/MS of acrylamide in foodstuffs

Sample (2 g), the internal standard (volume, corresponding to the concentration in sample 100 ng g^{-1}) and 5 mL of hexane were added into a 50 mL centrifuge tube, then the tube was vortexed. Distilled water (10 mL) and acetonitrile (10 mL) were added followed by the the QuEChERS extraction salt mixture (4 g anhydrous $MgSO_4$ and 0.5 g NaCl). The sample tube was shaken for 1 min vigorously and centrifuged at 4500 g for 5 min. The hexane layer was discarded, and 1 mL of the acetonitrile extract was transferred to a tube containing 50 mg of PSA-sorbent and 150 mg of anhydrous $MgSO_4$. The tubes were vortexed for 30 s and then the purified extract was analyzed by the ultra performance liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS).

The quantitative analysis of acrylamide was performed by LC-MS/MS using Acquity Ultra Performance LC system (Waters, Milford, MA, USA) coupled to a QTrap 5500 (AB Sciex, MA, USA). The separation of acrylamide was achieved with Luna 3u HILIC column (100×2.00 mm; Phenomenex, Torrance, USA). Methanol (6%) solution in acetonitrile acidified with 0.1% formic acid was used as a mobile phase (flow rate 0.25 mL min^{-1}, column temperature 40 °C and injection volume 10 µL). The conditions for the detection by MS/MS were as follows: 30 psi curtain gas (CUR), 5500 V ion spray voltage, 400 °C temperature, 40 psi ion source gas (GS1), 50 psi ion source gas (GS2), 60 V declustering potential (DP).

Acrylamide was quantified in food samples using the product ion m/z 55 (precursor ion m/z 72) for the analyte and m/z 58 (precursor ion m/z 75) for the internal standard (acrylamide-d3). The quantification was based on the peak area of analyte compared to that of the deuterated internal standard.

The limit of quantification for acrylamide was 10 µg kg^{-1}.

Quality control

The LC-MS/MS method for determination of acrylamide in food was validated. The following parameters were defined and tested: limit of quantification (LOQ), within-day precision, between-day precision and recovery. The laboratory has participated in the inter-laboratory proficiency testing organized in 2011 by FAPAS analyzing a biscuit test material and has received a Z-score -0.3. Furthermore, the analytical quality control sample was also used in each series of analysis to determine recovery of acrylamide during the sample preparation procedure.

Food consumption data collection

The comprehensive National Food Consumption database was developed in 2007-2009 and is updated from 2012-2013 to facilitate:

- risk assessment in relation to chemical and biological hazards in food,
- estimation of nutrient intakes by target population,
- development of scientifically based food policy documents.

During the first stage of the fieldwork in total 2000 individuals aged 19 to 64 years old and living in private households in Latvia were interviewed. Three age groups (19–35, 36–50, 51–64) were created. Information on food intake for each age group was collected using two non-consecutive 24 h recalls in combination with food frequency questionnaire. Additional data on sociodemographic and lifestyle characteristics were obtained in face-to-face interviewing process. Nutritional data were processed with the help of software for the storage and analysis of food consumption and composition data that was developed in frame of this project.

Design and the methodology of the food consumption survey was based on „European Food Consumption Survey Method (EFCOSUM)" recommendations (EFCOSUM, 2002), developed within the framework of the EU Programme on Health Monitoring to provide the common method for monitoring of food consumption in Europe in a comparable way.
Strictly standardized procedures were used in order to harmonise research methodology (including sample size, data analysis and data presentation) and to obtain data comparable with the data from other EU countries. For the quantification of portion sizes the food picture book was used. For presentation of the data, parameters of interest were mean, median, quartiles, P5 and P95.
The exposure assessment on the base of National consumption data has been conducted concerning chemical contamination risk and related nutrition risk to develop scientifically based conclusions and to ensure relevant risk management and communication activities.
At present very limited information about the content of acrylamide in Latvian origin food is available, therefore, the main objective of our study was to determine the content of this compound in the Latvian foods and to assess the average dietary acrylamide exposure of the Latvian population.

Results and Discussion

The acrylamide content in 435 samples of food manufactured in Latvia ranged from 8 to 2790 µg kg^{-1} of foodstuff. High standard deviation indicates significant variation of results obtained for individual product groups.
All food products samples were divided into three groups based on the mean value of acrylamide (<100 µg kg^{-1}, from 100 to 200 µg kg^{-1} and from 230 to 900 µg kg^{-1}). The highest mean acrylamide content falling into the range from 200 to 900 µg kg^{-1} was obtained for the following product groups: crisps, grain biscuits, gingerbread and coffee. The highest variations were observed in the group of crisps – from 42 to 1570 µg kg^{-1}. Relatively higher acrylamide content was determined in crisps with added flavour combinations and crisps produced, using potato varieties with high sugar content. The studies on mechanisms of the acrylamide formation (Taeymans et al, 2004) show that the increased presence of reducing sugars and fats significantly increases the acrylamide formation.
Our results were similar to those obtained by Eerola et al. (2007) in Finland (539 µg kg^{-1}), Murkovic (2004) in Austria (627 µg kg^{-1}) and EFSA (2011) (635 µg kg^{-1}). Slightly higher mean content of acrylamide in crisps was observed by Mojska et al. (2010) in Poland (904 µg kg^{-1}) and Sirot et al. (2012) in France (954 µg kg^{-1}). In all quoted studies the high acrylamide content variation within product groups was observed.
Mean acrylamide value in the range from 100 to 200 µg kg^{-1} was obtained for five product groups: bagels, biscuits, chocolate biscuits, savoury biscuits and puff pastry.

Table 1

Acrylamide amounts of various foods

Product description	N	Mean	S.D.	Median	Range, µg kg^{-1}
Bread					
Bread wheat	48	14	6	12	<10–36
Bread wheat with additives	11	29	16	31	10–54
Bread rye	77	48	16	47	14–87
Bread sweet sour	47	28	20	25	<10–133
Bread rye with additives	33	54	30	52	14–152
Rusk	16	65	27	57	27–93
Toast, rye bread	12	40	17	37	20–79
Snacks					
Crisps	55	564	517	303	42–1570
Bagels	22	162	128	119	39–588
Coffee					
Roasted	13	450	120	450	300–600
Soluble	4	900	110	920	790–980
Chicory	1	2790			2790
Pastry					
Biscuit	53	187	118	91	<10–1060
Biscuit, nuts	7	97	90	68	<10–279
Biscuits, grain	7	400	165	393	147–606
Biscuit, chocolate	6	141	122	115	23–341
Biscuits, savoury	8	125	123	66	<10–322
Butter biscuit, toffee	4	28	9	26	19–41
Gingerbread	2	238	59	238	196–280
Puff pastry	9	102	62	82	20–200

This group also showed rather high variation of the acrylamide content. Similar values have been reported by other authors (Murkovic, 2004; Rufian-Henares, 2007) with revealed acrylamide levels up to 2085 µg kg^{-1}. Biscuits and bagels produced with addition of different components, e.g. sesame, bran, poppies, and onions, contained higher levels of acrylamide.
The lowest acrylamide content was found in bread, bread products, biscuits with nuts and toffee. It should be noted that the acrylamide content in bread with addition of seed and dried fruit was significantly higher in comparison with samples of bread without any additives and this conclusion complies with the study of Taeymans, Wood et al, 2004 about the acrylamide content in lipid rich foods.

Table 2

Estimation of the acrylamide exposure for adults aged 19–35 years

Adults 19–35 years	Consumption, g per person per day			Acrylamide content, µg g^{-1}			Exposure assessment, µg per person per day		
Food products	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**
Rye bread	54.51	0.16	900.00	0.05	0.01	0.09	2.62	0.00	78.30
Rye bread with dried fruits	54.51	0.16	900.00	0.07	0.01	0.17	3.76	0.00	153.00
Sweet and sour bread	27.14	0.03	375.00	0.03	0.01	0.13	0.76	0.00	49.88
Wheat bread	52.57	0.05	1500.00	0.02	0.01	0.05	0.89	0.00	81.00
Cookies	11.29	0.03	213.70	0.16	0.01	0.61	1.76	0.00	129.50
Potatoes chips	9.90	0.01	136.88	0.56	0.04	1.57	5.58	0.00	214.90
Coffee	6.61	0.00	39.42	0.90	0.45	2.79	5.95	0.00	109.98

Table 3

Estimation of the acrylamide exposure for adults aged 36–50 years

Adults 36–50 years	Consumption, g per person per day			Acrylamide content, µg g^{-1}			Exposure assessment, µg per person per day		
Food products	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**
Rye bread	57.37	0.14	1600.00	0.05	0.01	0.09	2.75	0.00	139.20
Rye bread with dried fruits	57.37	0.14	1600.00	0.07	0.01	0.17	3.96	0.00	272.00
Sweet and sour bread	34.93	0.07	625.00	0.03	0.01	0.13	0.98	0.00	83.13
Wheat bread	58.31	0.07	750.00	0.02	0.01	0.05	0.99	0.00	40.50
Cookies	11.56	0.05	300.00	0.16	0.01	0.61	1.80	0.00	181.40
Potatoes chips	2.79	0.05	78.36	0.56	0.04	1.57	1.57	0.00	123.03
Coffee	9.06	0.01	231.73	0.90	0.45	2.79	8.15	0.00	646.53

Table 4

Estimation of the acrylamide exposure for adults aged 51–64 years

Adults 51–64 years	Consumption, g per person per day)			Acrylamide content, µg g^{-1}			Exposure assessment, µg per person per day		
Food products	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**
Rye bread	86.93	0.16	810.00	0.05	0.01	0.09	4.17	0.00	70.47
Rye bread with dried fruits	86.93	0.16	810.00	0.07	0.01	0.17	6.00	0.00	137.70
Sweet and sour bread	41.48	0.08	750.00	0.03	0.01	0.13	1.16	0.00	99.75
Wheat bread	51.41	0.05	470.14	0.02	0.01	0.05	0.87	0.00	25.39
Cookies	11.84	0.05	225.01	0.16	0.01	0.61	1.85	0.00	136.36
Potatoes chips	2.11	0.03	58.77	0.56	0.04	1.57	1.19	0.00	92.27
Coffee	8.49	0.01	70.52	0.90	0.45	2.79	7.64	0.00	196.75

Assessment of Latvian population exposure to acrylamide intake from food products

The mean daily consumption of seven product groups in which acrylamide intake was determined analytically and estimated exposure to dietary acrylamide intake for three Latvian individual age groups (adults 19–35 years, 36–50 years and 51–64 years) is presented in Table 2, 3 and 4. Based on the results of the actual daily consumption, out of all in the highest quantity. In the oldest population group (51–64 years) the consumption of bread was 179 g per person per day. The highest consumption of potato chips is observed for adults group 19–35 years (9.9 g per person per day). Mean consumption of coffee was equal for all groups of population with the high variability between individuals. The mean daily dietary acrylamide intake ranged from 17 μg per day in the adults group aged 19–35 years and 51–64 years to 18.7 μg per day in the adults group aged 36–50 years.

The acrylamide intake from food originated mainly in three product groups. In the Latvian population bread supplied 31% of the total dietary acrylamide intake in the group of adults aged 19–35 years, 33% in the group of adults aged 36–50 years and even up to 46% in the group of adults 51–64 years.

Potato chips on average supplied 16% of acrylamide in the adult population (19–64 years) with significant difference between age groups (from 32% in the group of adults aged 19–35 years to only 7% in the group of aged 51–64 years). The significant source of dietary acrylamide in the Latvian population was also coffee that supplied 40% of total dietary acrylamide intake in the adult population.

The calculated average dietary acrylamide exposure for the Latvian population aged 19–64 years is 0.26 μg kg^{-1} of body weight per day and this estimation is in good agreement with the WHO data showing that the average dietary acrylamide exposure for the general population ranges between 0.3 and 0.8 μg kg^{-1} of body weight per day (WHO, 2002). However it should be taken into account that not all foodstuffs that might contain acrylamide were regarded in our study including home-made products that might represent a major source of dietary acrylamide intake. Therefore the estimated acrylamide intake is probably lower than actual and further research is necessary. Besides it is disturbing to note that for the high consumer group the estimated exposure to dietary acrylamide intake exceeds even ten fold the estimate mean exposure for the total population (up to 3 μg kg^{-1} of body weight per day). This level coincide with the tolerable daily intake (TDI) for cancer estimated in other study (Tardiff et al., 2012) and therefore high dietary intake of acrylamide by certain consumers may potentially cause adverse health effects and certain activities should be made to diminish acrylamide levels in processed foods with elevated content of this compound in order to reduce the dietary risk to the human health for Latvian population.

Conclusions

A survey of the food products produced in Latvia was performed for determining the acrylamide contents in these products. The obtained data on content of acrylamide in foodstuffs was used to evaluate the dietary exposure estimates of the Latvian population. The highest level of acrylamide was detected in potato crisps and chicory coffee with average concentration 546 μg kg^{-1} and 2790 μg kg^{-1} respectively. In general, analysed product groups, bread was consumed crisps, biscuits and coffee were among the food products with the high level of acrylamide. Significant differences were observed in the acrylamide contents between different brands and within brands of the snacks and biscuits. The addition of seeds, fruits or vegetables in bread had an increasing effect on acrylamide content.

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