BREAD WASTE AND SAFETY OF REPROCESSED PRODUCTS

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Abstract
The aim of this study was to evaluate the bread consumption, bread waste, and practices of reprocessing it in household by conducting an online survey, and based on the results it were chosen two ways of reprocessing wheat and rye bread through toasting: in the form of slices and croutons. In order to obtain safe products for consumers, the toasting effect on acrylamide (AA) level of the end product and correlations between AA and colour parameters (L, a*, b*) were realized.*
Bread in the form of slices and croutons was toasted in the oven at 180°C for 10 and 20 min, respectively. AA content was determined by using GC-MS/MS method.
The AA level of fresh wheat and rye bread were 13.23 µg/kg, and 73.19 µg/kg, respectively. By toasting the bread, the AA level increased for wheat bread at 13.35-21.28 µg/kg for slices and to 23.77-50.71 µg/kg for croutons, while for rye bread the AA level increased at 77.03-106.66 µg/kg for slices, and to 138.58-634.62 for croutons, respectively. In the case of sliced bread, all samples are safe for consumption in regards to AA level which was under the benchmark level established by EU Commission 2017/2158. For croutons, the rye bread toasted for 20 min at 180°C exceeded the AA benchmark level. In the case of sliced bread, there is a linear correlation between the colour parameter L and the AA level (R^2= 0.7520-0.8774).*
The shelf life of bread can be extended by reprocessing, but the AA level increases by subjecting it to thermal processing. The AA level and colour parameters of reprocessed bread is influenced by the processing conditions, the type of flour used in the recipe, and also the way of cutting it.

Keywords: acrylamide, croutons, GC-MS/MS, rye bread, toasted bread, wheat bread

Received: 24.09.2020 Reviewed: 26.10.2020 Accepted: 16.11.2020

1. INTRODUCTION

Bread is a very consumed product in Romania being a part of our daily diet, Romania being among the top three bread consuming countries in Europe. Romanians consume over 95 kg bread per capita per year, while the European average is 60 kg per year (FRD, 2016). Due to the high amount of bread, this food product is particularly susceptible to wastage, having a short shelf life, losses during manufacturing taking place and also due to consumer preferences. Bread waste is a problem for bakery factories and in the same time for consumers. According to FAO (2019) the cause for consumer waste is the poor purchase and meal planning, the fact that consumers buy in excess, also confusion over labels, and poor in-home storing. In order to prevent consumer bread waste, as this product has a short shelf life, it can be reprocessed in households in different forms, one of it being toasting. During the toasting process, the chemical contaminant acrylamide, 2-propenamide (AA) can be formed. AA is a compound formed in starchy food products during the Maillard reaction as a result of the interaction between reducing sugars and amino acids, especially asparagine, at temperatures above 120°C and low moisture (EFSA, 2015). AA was categorized by the International Agency for Research on Cancer (IARC, 1994) as “a potential human carcinogen”, being a member of the Group 2A substances, considered to be a human neurotoxin. Even though the AA level in bread is low, due to the high bread consumption, this product is considered an important source of AA exposure. According to EFSA (2015), soft

bread contributes to adults AA exposure with a percent of 23% and estimates that adult chronic dietary exposure to AA is between 0.4 and 0.9 µg/kg b.w./day. In Romania, bread contributes to adults AA exposure with a percent of 26% and the adult chronic dietary exposure to AA is 0.9 µg/kg b.w./day (Negoiță and Culețu, 2016).
The AA formation in bread and similar products is influenced by the composition of the raw materials and ingredients used in the recipe, the formulation recipe, pH, also the baking parameters, temperature and time (Gündüz and Cengiz, 2015; Wang et al., 2017; Crawford et al., 2019). Wang et al. (2017) reported that fermentation reduces the AA formation, unleavened bread having a higher content of AA than the corresponding leavened bread. Forstova et al. (2014) studied the influence of different technological factors on the AA content of Czech leavened wheat-rye bread and wheat bread and showed that a lower AA content was found in breads with bigger sizes due to the smaller crust/crumb ratio, in bread leavened by natural rye sourdough due to a lower pH value of dough.
As AA is formed in many heat-processed-food products, the European Commission established mitigation measures and benchmark levels for reduction of this contaminant by implementing the Regulation 2017/2158. According to this regulation, the benchmark level for soft wheat-based bread was set to 50 µg/kg, for soft bread obtained from other cereals than wheat was set to 100 µg/kg, while for crispbread was set to 350 µg/kg.
Therefore, one of the aims of this study was to evaluate the bread consumption, bread waste, and practices of reprocessing it in household, and also the knowledge about the chemical contaminant formed when food products are subjected to temperature higher than 120°C by conducting an online survey, a questionnaire on which 453 respondents participated. Concerning the habits of reprocessing the bread, another aim of this research was to investigate the influence of toasting temperature and time of sliced bread and croutons on the AA level of reprocessed wheat and rye bread, and also to evaluate the correlation between the AA content and the colour parameters.

2. MATERIALS AND METHODS

Survey on bread waste
The online survey was realized by a questionnaire at which were included 453 participants that answered to questions focused on bread consumption and practices of reprocessing it, and also the knowledge about the chemical contaminant formed when bread is subjected to temperature higher than 120°C. Participants were part of the IBA Bucharest and other respondents who answered to the request to complete the survey. The questionnaire was launched in august 2020, via the Survio online platform. This questionnaire had 9 questions focused on evaluating a series of statements regarding the number of persons from the households (1 question), bread consumption (2 questions), bread waste (2 questions), practices of reprocessing and colour preferences (3 questions) and also about the knowledge of the compound formed during the Maillard reaction (1 question). Questions could have unique or multiple possible answers.

Food samples
For this study, widely consumed wheat and rye sliced bread purchased from a supermarket from Bucharest, Romania, were used.

Sample preparation
Both types of bread were cut in cubes of around 2 x 2 cm. Bread in the form of slices and cut in cubes (croutons) were toasted in the oven at 180°C for 10 and 20 min, respectively. Sample preparation for AA analysis was performed according to Mihai et al. (2020).

Reagents and reference standards
For AA determination, native AA of concentration 1004 µg/mL in methanol (99% purity) purchased from Restek (Benner Circle, Bellefonte, U.S.) and internal standard (IS) of labelled AA (1,2,3-^{13}C labelled AA) (99% purity) of concentration 1000 µg/mL in

methanol (+100 ppm hydroquinone) acquired from Cambridge Isotope Laboratories (Andover, MA, USA) were used. All solvents and reagents were of analytical grade, special for chromatography and were purchased from Merck (Darmstadt, Germany), LGC Promochem GmbH (Wesel, Germany), Alfa Aesar (Thermo Fisher Scientific, USA). Ultrapure water was obtained through a PURELAB Option-S7 and PURELAB Ultra Ionic system (Elga Labwater, High Wycombe, UK).

To assess the quality control, a certified reference material "acrylamide in rusk" (ERM®-BD274, BAM, Berlin, Germany) with the certified value of 74 µg/kg was used. In order to demonstrate the method's accuracy, a proficiency test (PT 3094/2019 – biscuit-cookie) launched by Food Analysis Performance Assessment Scheme (FAPAS) program was performed.

Preparation of reference solutions

Stock solutions of AA and IS (100 mg/L) were prepared in ultrapure water in amber vials. Working solutions of AA of concentration 10, 1 and 0.1 mg/L, respectively, and working solution of IS of concentration 10 mg/L were prepared from the stock solutions by dissolving in ultrapure water.

Preparation of calibration solutions

The calibration solutions were prepared according to the procedure described in a previous study (Negoiță and Culețu, 2016). Two calibration curves were realized in the range of 0.025– 5 mg/L based on the derivatized standard solutions and plotting the ratio of the peak area of 2-bromopropenamide (2-BPA) and 2-BP(^{13}C)A against the concentration of 2-BPA.

Acrylamide determination

To determine AA a gas chromatograph, type TRACE GC Ultra, coupled with triple quadrupole mass spectrometer (TSQ Quantum XLS) from Thermo Fisher Scientific (USA) was used. A capillary column based on polyethylene-glycol, TraceGOLD™ TG-WaxMS (30 m x 0.25 mm i.d. x 0.25 µm, Thermo Fisher Scientific, USA) was used as stationary phase. The mobile phase was helium.

The analyses were performed in the electron impact ionization operation mode, positive ($^{+}$EI), with the acquisition mode selected reaction monitoring (SRM) and the ion scanning mode- Product.

AA analysis was carried out according to the method described by Negoiță and Culețu (2016). Briefly, the following steps were followed: initial bread samples were dried at 50°C, for 3 hours and before and after drying, the moisture content was determined. The dried and toasted bread samples were then milled and homogenized in a Büchi professional mixer. AA was extracted in water and IS was added. Carrez solutions were used for deproteinization, and after this step samples were centrifugated. Bromine compounds were used for derivatization and the derivatized compound 2,3-dibromopropanamide (2,3-DBPA) obtained was extracted in a mixture of ethyl acetate: hexane (4:1, v/v). The extract was concentrated first with a vacuum evaporation system (40°C, 156 – 90 mBar), and then to dryness under a nitrogen stream. A chromatographic column filled with florisil and sodium sulphate was used for purification. The 2,3-DBPA derivative was eluted with acetone, then it was concentrated till dryness and the residue was redissolved in ethyl acetate and triethylamine. The final extract of 2-BPA was injected to GC-MS/MS.

AA quantification was performed by the internal standard method. AA content was expressed as mean ± standard deviation (sd).

Method performance

The method used for acrylamide quantification in bread, biscuits and other bakery products was described by Negoiță et al. (2020). The recovery of the procedure was in the range of 97% - 105%. The relative standard deviations (RSD) for repeatability and reproducibility were 0.4– 4.9% and 2.23– 5.10%, respectively.

Colour analysis

For all analysed samples it were also determined the colour parameters (L^*, a^*, b^*) by using a Konica Minolta spectrophotometer. Instrument calibration was done with black and white pads supplied by the manufacturer. The colour of the samples was measured using illuminate D65 with an angle of view of 10°.

The L^* parameter measures the object luminance intensity on a scale from 0 to 100, where 0 represents black and 100 white, a^* parameter represents the colour position of the object on a scale from pure red and pure green, where pure green is -127, and pure red is +127, while b^* parameter represents the position of the object on a scale colour of pure blue and pure yellow, where pure blue is -127 and pure yellow is +127. For each sample, measurements were made on 10 different points, and the mean value was determined.

Statistical analysis

All analyses for AA determination were performed in duplicate and the results were subjected to statistical analysis by calculating the mean content and the sd. Results were expressed in µg/kg. In the case of colour parameters, analyses consisted in 10 measurements made in different points and results were expressed as value ± sd.

3. RESULTS AND DISCUSSIONS

Bread waste survey

In order to determine the consumers perception regarding bread waste and bread consumption, the results reported by the questionnaire realized on the online platform Survio were analysed.

The results of the survey regarding the number of persons from the households showed that 6.2% of households are formed of 1 person, 31.8% of 2 persons, 33.3% of 3 persons, while 28.7% are formed of more than 3 persons.

From all participants, 95.4% consume bread purchased from the market, while only 4.6% prepare it on the household. In terms of buying habits, the results showed that 27.2% of respondents buy bread daily, 35.3% once at 2 days, 28.3% once per week, while 9.3% gave other answers.

Regarding the bread consumption in household, the results provided showed that only 37.5% of respondents to the survey consume all bread that they buy. From the participants who don't consume all bread, 49.7% reuse it, 46.6% throw it, and 13% keep it on the freezer. As shown in figure 1, the results of the survey revealed that respondents reuse the bread in different ways, like bread crumbs, croutons, sliced bread, toasted, as an ingredient or they gave other answers. Most of the responders who gave other answers indicated that they use it as food for animals. Taking into consideration the answers to the survey, we decided to reprocessed the bread in the form of croutons and sliced bread by toasting it in an oven.

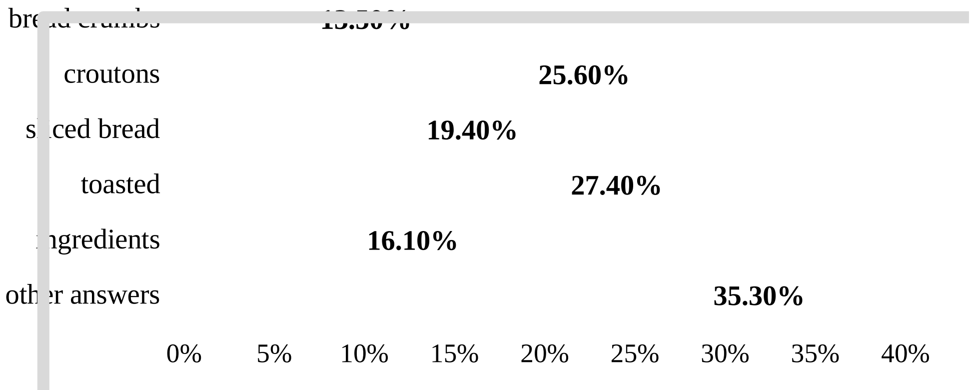

Figure 1. Respondents preferences regarding bread reprocessing in households

Regarding the colour preferences of bread baked in the oven, 57% of respondents choose a certain colour, while 43% don't have a preference. Out of the 57% respondent who prefer a certain colour, 7.1% prefer the bread to be dried after oven baking but without major colour changes, 39.2% likes golden colour, 39.0% prefer golden-brown colour, 10.4% prefer the bread to have a brown crust, while 4.3% of respondents have other preferences.
As well, respondents were asked about the knowledge of the chemical contaminant with possible carcinogenic effects that is formed when food products are subjected to temperature higher than 120°C, and 29.4% of them knew about it, while 70.6% answered that they don't know it.

Quality control
The calibration curves were linear (correlation coefficient R > 0.99) in the range of 0.025 – 0.5 mg/L and 0.4 – 5 mg/L. Limit of detection and limit of quantification were 1.23 µg/kg and 3.70 µg/kg, respectively.
For each set of analysis, the quality control "acrylamide in rusk" with the certified value of 74 µg/kg was used. The proficiency test result for biscuit-cookie (z-score= -0.1) suggest that the method for AA quantification is accurate.

Influence of toasting on AA level of bread
Home-cooking practices have an important influence on the AA daily intake. For this study, wheat and rye bread were chosen, one being part of the soft bread and the other being part of bread obtained from other cereal than wheat. Wheat and rye sliced bread were toasted in an oven at the temperature of 180°C for 10 and 20 min, respectively and the final products were analysed to determine the AA level formed.
The sliced wheat bread had initially an AA content of 13.23 µg/kg, under the benchmark level of 50 µg/kg set by the European Commission (EU) 2017/2158.
As it can be seen from table 1, by toasting the wheat bread in the form of slices, the AA content increased after 10 min of toasting at 180°C with a small percent, reaching the level of 13.35 µg/kg, and after 20 min it increased at 21.28 µg/kg, higher with 60.85% than the initial AA content.
In the case of wheat bread cut in the form of croutons, the AA content increased after 10 min of toasting at 23.77 µg/kg, while after 20 min it increased at 50.71 µg/kg, being higher with a percent of 79.67% for 10 min, and 283.30% for 20 min, respectively than the untoasted bread sample.
Even though the AA level was higher for the toasted bread in the form of slices and croutons than the initial level of bread, the content was under the benchmark level of 350 µg/kg set by the European Commission (EU) 2017/2158 for crispbread. For rye bread, the initial AA content determined was 73.19 µg/kg, under the benchmark level of 100 µg/kg established by the Commission Regulation (EU) 2017/2158 for soft bread obtained from other cereals than wheat. By toasting the sliced rye bread for 10 min, the AA level increased at 77.03 µg/kg, while by toasting it for 20 min it increased at 106.66 µg/kg, being higher with 5.35% and 45.73%, respectively than the untoasted samples. In the case of croutons, the AA level increase was much higher, after 10 min of toasting at 180°C, the AA content reached a

Table 1. Acrylamide content of the bread samples analysed

Toasting conditions		Rye Bread		Wheat bread	
		AA content (mean ± sd), µg/kg	Sample codification	AA content (mean ± sd), µg/kg	Sample codification
Initial/untoasted		73.19 ± 2.09	A_o	13.23 ± 0.64	B_o
Slices	10′/180°C	77.03 ± 0.93	A_{1s}	13.35 ± 0.20	B_{1s}
	20′/180°C	106.66 ± 1.15	A_{2s}	21.28 ± 0.40	B_{2s}
Croutons	10′/180°C	138.58 ± 0.45	A_{1c}	23.77 ± 0.56	B_{1c}
	20′/180°C	634.62 ± 10.19	A_{2c}	50.71 ± 0.56	B_{2c}

level of 138.58 μg/kg (higher with 89.34%), while after 20 min it was determined an AA content of 634.62 μg/kg (higher with 767.09%). For the rye croutons toasted for 20 minutes, the AA level exceeded the benchmark level of 350 μg/kg for crispbread set by the regulation in force by approximately 2 times.
The results revealed that for both kind of bread, wheat and rye bread, toasted in the form of slices and croutons, the AA content was higher in the case of croutons. This could be explained by the fact that the moisture content of the product in the form of croutons drops faster due to the higher surface exposed to temperature and creates a favourable environment for AA to form. The AA content in crust is higher than in the crumb (Wang et al., 2017) and in the case of croutons, the crust has a higher percent comparing to sliced bread.
From this study it can be noticed that the AA content was higher for rye bread than for wheat bread. This could be explained by the fact that rye flour contains more asparagine than wheat flour, one of the precursors for AA formation. In the study realized by Prygodzka et al. (2015) it was shown that the asparagine content of wheat flour with a 100% extraction rate was 77.5 mg/kg, comparing with rye flour with a 100% extraction rate which had a content of 312.9 mg/kg. Capuano et al. (2009) reported a higher AA content for rye bread, comparing to wheat bread. Similar results were obtained by Gündüz and Cengiz (2015) who found a higher AA content for rye bread than for the wheat bread.
The results presented in our study are similar with the ones obtained in the research conducted by Raatikainen et al. (2015) who showed that by toasting the rye bread for 90–210 s, the AA level increased from 50- 350 μg/kg d.w. to 50- 830 μg/kg d.w., the AA content being higher in samples toasted for a longer time. By subjecting the wheat bread to toasting, the AA content increased from undetectable (<40 μg/kg) to 210 μg/kg d.w.

Correlation between the AA level and the colour parameters

For all bread samples analysed it were determined the colour parameters. Colour in bread samples is a result of the Maillard reaction and it is influenced by the content of reducing sugars of the raw materials, processing conditions, also by the moisture content. In order to determine the correlation between the AA level formed in toasted bread and the colour parameters, the regression lines were drawn, and the regression equations and correlation coefficients values between these parameters were calculated for each type of toasted bread.
By toasting the bread, the lightness of the bread (L^*) decreased with increasing toasting time, the L^* values of the toasted bread being lower than that of the fresh bread. For bread cut in the form of cubes, there is a linear correlation between the colour parameters L^*, a^*, b^* and the AA level: R^2= 0.7520 - 0.8774.

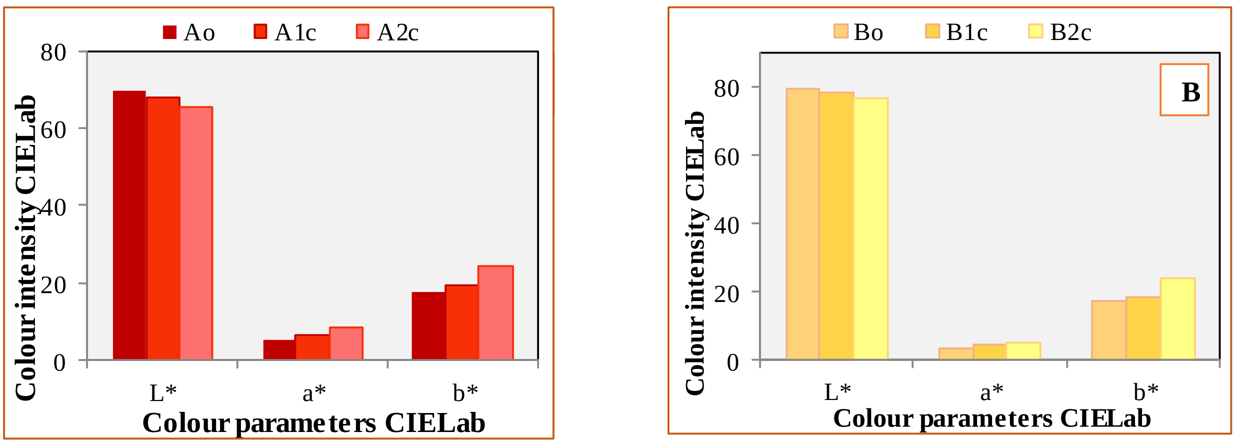

Figure 2. Colour parameters *L, *a**, *b** for croutons of rye bread (A) and for croutons of wheat bread (B)**

As it can be seen in figure 2, for the rye and wheat bread in the form of croutons the $L*$ values decreased by increasing the toasting time, while for $a*$ and $b*$ parameters the values slightly increased during toasting for 10 and 20 min, respectively. Similar results were reported by Capuano et al. (2009) who showed that by toasting at different temperatures and different times a bread crisp model system made of different flour types (wheat, rye and whole-wheat flours) the $L*$ value decreases with increasing toasting time and temperature, while $a*$ and $b*$ values increased.

4. CONCLUSIONS

Based on the results of the online survey realized, two ways of reprocessing the bread were chosen, like croutons and sliced toasted bread. Even though the shelf life of reprocessed bread compared to the fresh bread can be extended, the AA level can increase by subjecting the bread to thermal processing.

In the case of sliced and croutons wheat bread and sliced rye bread, toasted at 180°C for 10 and 20 min, respectively, none of the samples exceeded the AA benchmark level of 350 µg/kg established by EU Commission 2017/2158, being safe for consumption. The rye bread in the form of croutons toasted for 20 min at 180°C exceeded the benchmark level established by the regulation in force.

The AA level of reprocessed bread is influenced by the baking conditions, the composition of the raw materials and ingredients used in the recipe, and also the way of cutting it.

ACKNOWLEDGMENTS

This study was achieved through Core Programme (PN 19 02), with the support of the Ministry of Research and Innovation (MCI), contract 22N/2019, project PN 19 02 03 01.

5. REFERENCES

1. FRD Center – Food market in Romania, 2016 https://www.dutchromaniannetwork.nl/wp-content/uploads/2017/01/Food-Report-Romania-2016.pdf
2. FAO. 2019. The State of Food and Agriculture 2019. Moving forward on food loss and waste reduction. Rome. Licence: CC BY-NC-SA 3.0 IGO. ISBN 978-92-5-131789-1
3. European Food Safety Authority (EFSA). 2015. Scientific opinion on acrylamide in food. EFSA Panel on contaminants in the food chain (CONTAM). EFSA Journal, 13(6), 4104.
4. International Agency for Research on Cancer (IARC), 1994. Monographs on the Evaluation of Carcinogenic Risks of Chemicals to Humans, Vol. 60, 389, Lyon, France.
5. Negoiță, M., Culețu, A., Application of an accurate and validated method for identification and quantification of acrylamide in bread, biscuits and other bakery products using GC-MS/MS system. Journal of Brazilian Chemical Society, **27**(10), 2016, 1782-1791.
6. Gündüz C.P.B., Cengiz M.F., Acrylamide contents of commonly consumed bread types in Turkey, International Journal of Food Properties, **18**, 2015, 833–841.
7. Wang S., Yu J., Xin Q., Wang S., Copeland L., Effect of starch damage and yeast fermentation on acrylamide formation in bread. Food Control, **73**, 2017, 230-236.
8. Crawfors L.M., Kahlon T.S., Chiu M.C.M,m Wang S.C., Friedman M., Acrylamide content of experimental and commercial flatbreads. Journal of Food Science, **84**(3), 2019, 659 – 666.
9. Forstova, V., Belkova, B., Riddellova, K., Vaclavik, L., Prihoda, J., Hajslova, J., Acrylamide formation in traditional Czech leavened wheat-rye breads and wheat rolls, Food Control, **38**, 2014, 221-226.
10. European Commission (EU) (2017). Commission Regulation 2017/2158 of 20 November 2017 establishing mitigation measures and benchmark levels for the reduction of the presence of acrylamide in food. Official Journal of the European Union. L304, 24–44. Available from: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32017R2158&from=EN
11. Mihai A.L., Negoiță M., Horneț G.A., Assessment of the acrylamide level of cereal-based products from Romania market in accordance with Commission Regulation (EU) 2017/2158. The Annals of the University

Dunărea de Jos Galați, Fascicle VI-Food Technology, **44**(1), 2020, 104-117.

12. Negoiță M., Mihai A.L., Horneț G.A., Duță D.E., Development of SPE clean-up procedure for acrylamide determination from potato-based products by GC-MS/MS, Open Agriculture, **5**(1), 2020, 305-316
13. Przygodzka M., Piskula M.K., Kukurová K., Ciesarová Z., Bednarikova A., Zieliński H., Factors influencing acrylamide formation in rye, wheat and spelt breads. Journal of Cereal Science, **65**, 2015, 96-102.
14. Capuano E., Ferrigno A., Acampa I., Serpen A., Açar Ö. Ç., Gökmen V., Fogliano V., Effect of flour type on Maillard reaction and acrylamide formation during toasting of bread crisp model systems and mitigation strategies, Food Research International, **42**, 2009. 1295–1302.
15. Raatikainen M., Kronlöf E., Salovaara H., Koivisto P., Acrylamide in rye bread - A risk in Finnish diet? Abstract/ Toxicology Letters, 238S, 2015, S56- S383.