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Health concerns associated to tropane alkaloids in maize food products in Serbia

Ljilja Torović [a,b,*], Vojislava Bursić [c], Gorica Vuković [d]

[a] University of Novi Sad, Faculty of Medicine, Department of Pharmacy, Hajduk Veljkova 3, 21000, Novi Sad, Serbia
[b] University of Novi Sad, Faculty of Medicine, Center for Medical and Pharmaceutical Investigations and Quality Control, Hajduk Veljkova 3, 21000, Novi Sad, Serbia
[c] University of Novi Sad, Faculty of Agriculture, Trg Dositeja Obradovića 8, 21000, Novi Sad, Serbia
[d] University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11000, Belgrade, Serbia

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ABSTRACT

Following the implementation of the European regulation limiting the presence of tropane alkaloids in certain foods, a survey was conducted in Serbia on 103 maize products (grits, polenta and semolina) to determine atropine and scopolamine content using liquid chromatography with tandem-mass spectrometry analysis (LC-MS/MS). The probability of exceeding the Acute Reference Dose (ARfD; 0.016 μg/kg bw/day) of the sum of atropine and scopolamine by consuming these products was tested. Overall, across age categories - children, younger and older adolescents, and adults, the group ARfD was exceeded by 21.4%, 17.5%, 11.7% and 11.7% of the samples, with maximum exposure reaching as much as 19-, 13-, 9- and 9-fold the group ARfD, respectively. Nevertheless, polenta could be the most favorable dietary option (17.9% of positive samples, 7.7% resulting in excessive exposure in children, reaching a maximum of 1.4-fold the group ARfD). According to the reported findings, adverse health effects of tropane alkaloids cannot be ruled out. The Margin of Exposure, founded on a clinically significant acute effects dose established by FAO/WHO, ranged from 1194 to 2381 (mean) and from 28 to 56 (95th percentile) across age categories. These estimates should certainly draw the attention of food authorities and nutritionist, particularly in the case of highly sensitive populations with contraindications and high consumers of corn products, such as coeliac patients.

1. Introduction

Recently conducted special Eurobarometer survey related to food safety has shown that the highest number of respondents across the European Union express concerns related to the health impact of food (20%), followed by food contaminants (17%), which topped the list of risks associated with food in five countries. Nearly half of Europeans showed equal concern about healthy diet and food risks, but as many as 41% took it for granted that the food sold is safe [1]. According to the Food and Agriculture Organization and World Health Organization, the principal objectives of a food control system are: "protecting public health by reducing the risk of foodborne illness; protecting consumers from unsanitary, unwholesome, mis-labelled or adulterated food; and contributing to economic development by maintaining consumer confidence in the food system and providing a sound regulatory foundation for domestic and

* Corresponding author. University of Novi Sad, Faculty of Medicine, Department of Pharmacy, Hajduk Veljkova 3, 21000, Novi Sad, Serbia.
E-mail address: ljilja.torovic@mf.uns.ac.rs (L. Torović).

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international trade in food". The typical building blocks of such a system are food law and regulations, food control management, inspection and laboratory services, and information, education and communication [2].

Emerging food contaminants pose a unique challenge in terms of their incorporation into food regulations, most often due to the lack of toxicological and/or occurrence data. The EU Commission adopted Recommendation 2015/976/EU on the monitoring of the presence of tropane alkaloids (TA) in food [3], followed by the Regulation 2016/239, limiting the atropine and scopolamine concentration in cereal-based foods for infants and young children [4], and then the Regulation 2021/1408 [5], amending the Regulation 1881/2006 [6] by establishing maximum levels of TA in processed cereals and cereal products. Consequently, the necessary regulations are in place. Food control management is responsible for determining "why" to test, whereas laboratories must consider "what" and "how" (https://www.newfoodmagazine.com/article/23154/analytical-governance-food-industry), and ensure the validity of their results, to make certain that the testing is relevant to decision-making. The Commission Recommendation 2015/976 stipulates certain requirements for the analytical methods [3], and although a standardized method for the analysis of TA in food has not yet been established, currently available validated state-of-the-art liquid chromatography with tandem-mass spectrometry (LC-MS/MS) methods are able to achieve the LOQ of 1 μg/kg or less [7]. Hence, the necessary laboratory services are in place. When it comes to the national food safety systems in developing countries, communication block often shows numerous shortcomings related both to internal and external communication.

TA contamination of food has been associated with either food plants containing inherent TAs or contamination of food with co-harvested weeds producing TAs as secondary metabolites, in particular, Brugmansia, Datura and Hyoscyamus species (Solanaceae plant family) [8]. Weed seeds could be accidently mixed with edible plants during harvest or processing, especially with grains, cereals and pseudocereals such as linseed, soybean, millet, sunflower and buckwheat, because of the similarities in their density, size, and shape [9]. It has been shown that the presence of a single undetected TA-contaminated seed per million of crop seeds might pose health concerns. Importantly, the presence of seeds might be reduced by the raw material source selection and improved grain sorting and cleaning, *i.e.*, risk management measures not applicable after grain milling. It should be noted that organic agriculture, due to a less strict weed management, might lead to an increased in-field presence of TA-producing plants alongside with crops [10].

TA share a common core tropane skeleton, a two-ringed (pyrrolidine and a piperidine) structure characterized by structural variations resulting from the esterification of tropine with a variety of acids, leading to the creation of more than 200 compounds found in plants. Datura-type TA most frequently found in food in the highest concentrations are esters of tropic acid, hyoscyamine and scopolamine. Although the acid's asymmetric α-carbon allows the formation of two stereoisomers, (−)-hyoscyamine and (−)-scopolamine were the ones predominantly formed in biosynthetic pathways. The most important TA in food is atropine, which is a racemic mixture of (±)-hyoscyamine. Plant extracts containing TA have been used in human medicine throughout history [11]. However, the worldwide occurrence and investigations of poisoning due to TA contamination of medicinal plants (herbs and herbal teas) have emphasized the crucial importance of good agricultural and collection practices [12]. As humans are highly sensitive to anticholinergic action of TA, even miniscule amounts might trigger severe poisoning, as evidenced in several TA intoxication incidents reported recently [8]. TA contamination of cereals and soybean blend product was the cause of multiple serious and fatal food poisoning incidents in Uganda during 2019 [13]. In March 2021, an alert notification was reported based on food poisoning caused by deeply frozen spinach puree. Over 100 people reported various symptoms (nausea, dizziness, dry mouth, malaise, blurred vision, mydriasis) to the Slovak National toxicological information center (https://ec.europa.eu/food/safety/rasff_en). Based on the results for decreased heart rate in a human volunteer study [14], the European Food Safety Authority (EFSA) established the group acute reference dose (ARfD) for the sum of atropine and scopolamine at 0.016 μg/kg bw, approximately two orders of magnitude lower than the lowest single therapeutic doses [9]. Based on the dose-response relationship established for TA-induced reduction of salivary secretion in the same human volunteer study [14], the FAO and WHO Expert committee established a clinically significant minimal acute effect dose of 1.54 μg/kg bw [7].

Cereals and cereal products constitute staple food for the majority of the populations worldwide. In Serbia and throughout Balkan region, maize milling products have been predominantly used for the preparation of ethnic meals, namely "kačamak" (a porridge-like dish), "proja", "cicvara". Nowadays, they have been promoted in traditional food campaigns and hence their popularity is on the rise. Additionally, due to their gluten-free nature, they are often included in special dietetic foods intended for the population suffering from coeliac disease [15]. In marketing year 2021/22, corn production in Serbia reached around 6 million metric tons, sufficient to meet domestic demand and allow for substantial corn exports (Serbia ranks among the top ten largest exporters of corn in the world) [16].

This study presents a risk assessment based on the findings of a previous occurrence study conducted on maize milling food products sampled in the Serbian market in 2021 [17] in order to perceive their contamination with the TA (referring to atropine and scopolamine). The main objective of the study was to elucidate the potential health risks posed by the consumption of TA-contaminated maize products by children, adolescents and adults, as well as to provide valuable information to food safety authorities and thus foster achievement of food safety system objectives.

2. Material and methods

2.1. Samples and sample preparation

Samples of maize grits (33), semolina (31) and polenta (39) were collected from food retail stores during 2021, in unopened original packages as available to consumers. The sample collection encompassed all major brands and producers present in the Serbian market. Samples were prepared using the QuEChERS method for atropine and scopolamine extraction. Additional information is available in previously published work [17].

2.2. LC-MS/MS analysis

The content of atropine and scopolamine was determined using a high-performance liquid chromatography (HPLC) system – the Agilent 1290 Infinity II, coupled with an Agilent 6495 LC/TQ triple quadrupole mass spectrometer equipped with a jet stream technology ion source (AJS ESI) (Waldbronn, Germany). The method performance was fully characterized. Briefly, spiking of tropane alkaloids standards into grits, polenta and semolina matrices in amounts from 1 to 20 μg/kg (corresponding to the method linearity range) resulted in 86.8 ± 14.6, 89.1 ± 16.1 and 85.3 ± 15.8% recoveries (mean ± standard deviation), respectively. The limit of detection was calculated by MassHunter software, based on the signal-to-noise ratio (0.1 μg/kg) and the limit of quantification was set at 1 μg/kg (the lowest calibration level) for both atropine and scopolamine, which was in compliance with the Commission Recommendation 2015/976 [3]. Additional information is available in previously published work [17].

2.3. Data processing

For data analysis and overall considerations related to the collected maize milling products in their entirety, the samples were further divided into three groups: semolina, grits and polenta. Each of these has traditionally been used in the preparation of different meals. The quantified amounts of the alkaloids were evaluated against criteria defined by EU Regulation 2021/1408 [5], setting maximum levels of TA allowed in processed cereals and various cereal products, including maize. Furthermore, the ratio between the amounts of atropine and scopolamine quantified in the same sample provided an insight into the possible origins of alkaloid-contamination. Most of the analytical results were left-censored: overall, 65.0 and 73.8% of them were reported as below the LOD, and 3.9 and 3.9% as below the LOQ, for atropine and scopolamine, respectively. Considering that TA are naturally occurring toxicants, and as such are likely to be present in food, prior to the exposure assessment, the left-censored data were treated by the substitution method, as indicated by the EFSA [18] and recommended by the FAO and WHO [19]. This substitution method means that, under the lower-bound approach (LB), all results < LOD and < LOQ were replaced by zero; and, at the upper-bound (UB), the results < LOD were replaced by the LOD and those < LOQ by the LOQ.

2.4. Input data

The consumption amounts of maize products relevant to acute effects (100 g for children, 133 g for adolescents and 152 g for adults, per day) were derived from the EFSA PRIMo model v.3 [20]. All of the values obtained for acute alkaloid intake were adjusted for bodyweight for different age categories, children (corresponding to ′other children' in EFSA categorization; 3–10 years, 23.1 kg), younger (11–14 years, 43.4 kg) and older adolescents (15–18 years, 61.3 kg, respectively) and adults (>18 years, 70 kg) [21].

2.5. Exposure and risk assessment

The exposure of specific population groups was calculated using a deterministic approach, by multiplying the measured alkaloid levels with the consumption amounts of maize products relevant to acute effects, and further adjusting for bodyweight. Exposure to the sum of atropine and scopolamine was calculated based on the sum of atropine and scopolamine in the same sample. Additionally, exposure to individual alkaloids was assessed to determine their individual contribution.

The toxicological risk assessment of maize products was based on comparison of the estimated exposure with the group ARfD of 0.016 μg/kg bw established by the EFSA [9]. Whether the ARfD could possibly be exceeded was evaluated on a case-by-case basis for each product under assessment. The proportion of products causing acute exposure of each age group of consumers above the ARfD, as well as mean and high level of exposure were calculated. Since the number of the samples in separate product groups was limited (from 31 to 39), high levels of contamination were represented by the average of the last quartile, which was considered more statistically robust than 95th percentile.

Additionally, the margin of exposure (MOE) approach was applied, as demonstrated by FAO and WHO [7]. The MOE was calculated using the formula MOE = MAED/CAE, where: MOE represents the margin of exposure (unitless), MAED is the clinically significant minimal acute effect dose (1.54 μg/kg bw) [7], CAE represents the combined acute exposure to atropine and scopolamine (μg/kg bw).

2.6. Statistical analysis

All results were processed using MS Office Excel 2019. Statistical analysis was conducted in order to determine the significance of differences (level of significance: $p < 0.05$) between groups of maize milling products in relation to alkaloid occurrence rates (Chi-square analysis) and concentrations (Analysis of variance, ANOVA). The analysis was carried out using Statistica TIBCO, Version 14.0.015 (TIBCO Software Inc.).

3. Results and discussion

3.1. Considerations related to the alkaloid-contamination of maize milling products

Several aspects of the contamination data not comprised by the occurrence study of Vuković et al. [17] have been considered to provide a more comprehensive analysis of the obtained data relevant for the current study. Overall, with regard to atropine, 35.0% of the results were above the LOD. However, grits (45.5%) and semolina (45.2%) showed a statistically significant higher frequency of occurrence compared to polenta (17.9%). The significance of this difference was further confirmed when the mean contamination levels were considered (2.5 μg/kg overall; 2.6, 5.2 and 0.4 μg/kg in grits, semolina and polenta, respectively; $p = 0.001$ and $p = 0.022$). Additionally, semolina outweighed grits by a double mean contamination level, primarily due to a couple of samples with extreme atropine concentrations (Fig. 1). Although a high degree of co-occurrence was observed, atropine was present more frequently and at higher levels than scopolamine (overall 26.2% and 0.7 μg/kg), with relative concentration ratios of 3.1 ± 1.2 (overall), 3.8 ± 0.8 (grits), 2.8 ± 1.4 (semolina) and 2.2 ± 1.4 (polenta), indicative of *Datura stramonium*, known as one of the major Datura species and a common source of TA contamination in the warm temperate zone of southern Europe [7].

As of September 2022, the sum of atropine and scopolamine in maize milling product shall not exceed 5.0 μg/kg, whereas the maximum limit for processed cereal-based foods has been in force since 2021, limiting the atropine and scopolamine content to 1.0 μg/kg of each alkaloid [5,6]. The Serbian survey showed excessive contamination in 18.4% of the tested maize milling products, with 15.5%/3.9% of them with atropine/scopolamine alone exceeding the limit. The distribution of overly contaminated samples by product type revealed a dominance of grits (30.3%), followed by semolina (20.5%) and far behind polenta samples (2.6%). The European survey of a broad range of plant-derived foods, including cereals and cereal products, recorded a variable composition, coverage and levels of contamination with TA (atropine and scopolamine were the ones most frequently present, comprising 83% of the reported content in cereals) [22,23]. The proportion of contaminated corn flours (23.7%) was lower than reported for the Serbian samples, as well as the determined concentrations (sum of atropine and scopolamine <1 μg/kg). The main findings of other studies relevant to cereals were the following: cereal-based products (including maize flour) collected internally from Nestlé factories in Asian and African countries were found free of atropine and scopolamine, with the exception of one rice-based product [24]; wheat, rye, wheat-rye and multi-grain bread from the Netherlands showed the absence of TA [25]; in buckwheat, wheat, soy, buckwheat flour, buckwheat noodle, amaranth grain, chia seeds and peeled millet from Spanish market, TAs were not found above the detection limits [26]; in buckwheat-derived organic foods (flours, pasta and bakery products) from Italy, TAs were detected in 3 out of 26 samples, only in one in the amount relevant in terms of food safety [10]. Specifically for cereal-based foods for infants and young children, TAs were found in 22% of the samples from the Netherlands, at mean levels decreasing from 3.9 μg kg in 2011 to 0.4 μg kg in 2014 [27], while among samples from Spain, France and Italy, classified as pap, biscuits, crackers, snack and grissines, only one sample of biscuits composed mainly of buckwheat tested positive [28].

3.2. Considerations related to health risk assessment

3.2.1. Acute exposure to TA in relation to the group ARfD

The acute dietary exposure to TA by consumption of maize products across all age categories, was compared with the TA group ARfD (0.016 μg/kg bw for the sum of atropine and scopolamine), as shown in Fig. 2. The highest acute dietary exposure to TA was observed among children, nearly 19-fold the group ARfD in case of the most contaminated semolina sample (atropine:scopolamine ratio 5.8:1), or up to 6-fold in case of grits. If consumed by children, as many as 22 samples would exceed the group ARfD (11 grits + 8 semolina +3 polenta). In other age groups the consumption of the products under assessment was likely to cause excessive acute exposure in 18 cases (younger adolescents; 10 grits + 8 semolina) and 12 (older adolescents and adults; 7 grits + 5 semolina). The probability of exceeding the group ARfD, calculated by dividing the number of cases where acute exposure was above the group ARfD by the total number of cases (number of samples), was 21.4% overall, 33.3% among grits, 25.8% among semolina and 7.7% among polenta samples consumed by children. Semolina and grits provided TA in amounts more than 9- and 3-fold the group ARfD even for adult and older adolescent consumers. The choice of LB or UB approach had no influence on the probability of surpassing the group ARfD (more details in section 3.3.), while the mean exposure estimates were very close in both approaches. Based on the UB estimates, the overall range of mean exposure to the sum of TA across age categories varied from 42.4 to 92.6% of the group ARfD. This was 49.3–98.4% in case of grits and 89.6–178.6% in case of semolina samples, with the upper end estimates corresponding to children. The overall (samples in their entirety) estimates of high acute dietary exposure, calculated as either P95 or the mean of the last quartile, were virtually the same: approximately 3.4-, 2.4-, 1.7- and 1.7-fold the group ARfD, in case of children, younger and older adolescents, and adults, respectively. High exposures (the last quartile mean) related to grits were very close to the overall ones, while semolina

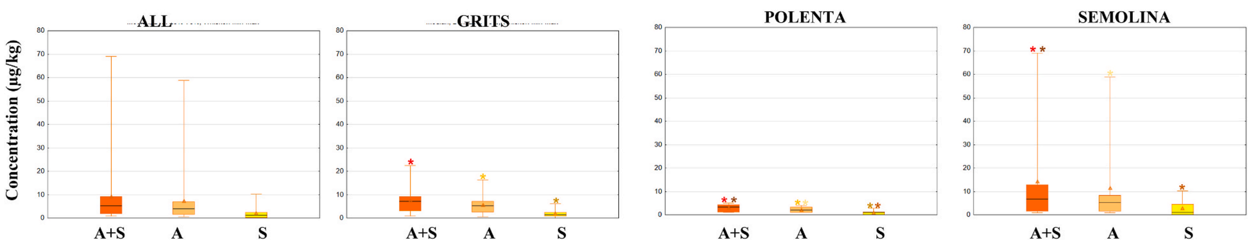

Fig. 1. Box-Whisker plot of concentrations of tropane alkaloids (sum of atropine and scopolamine (A + S), atropine (A) and scopolamine individually (S)) present in maize milling food products. Whiskers extending from minimum to maximum, □ interquartile range, − median, Δ mean. Colored stars denote statistically significant concentration differences between product groups.

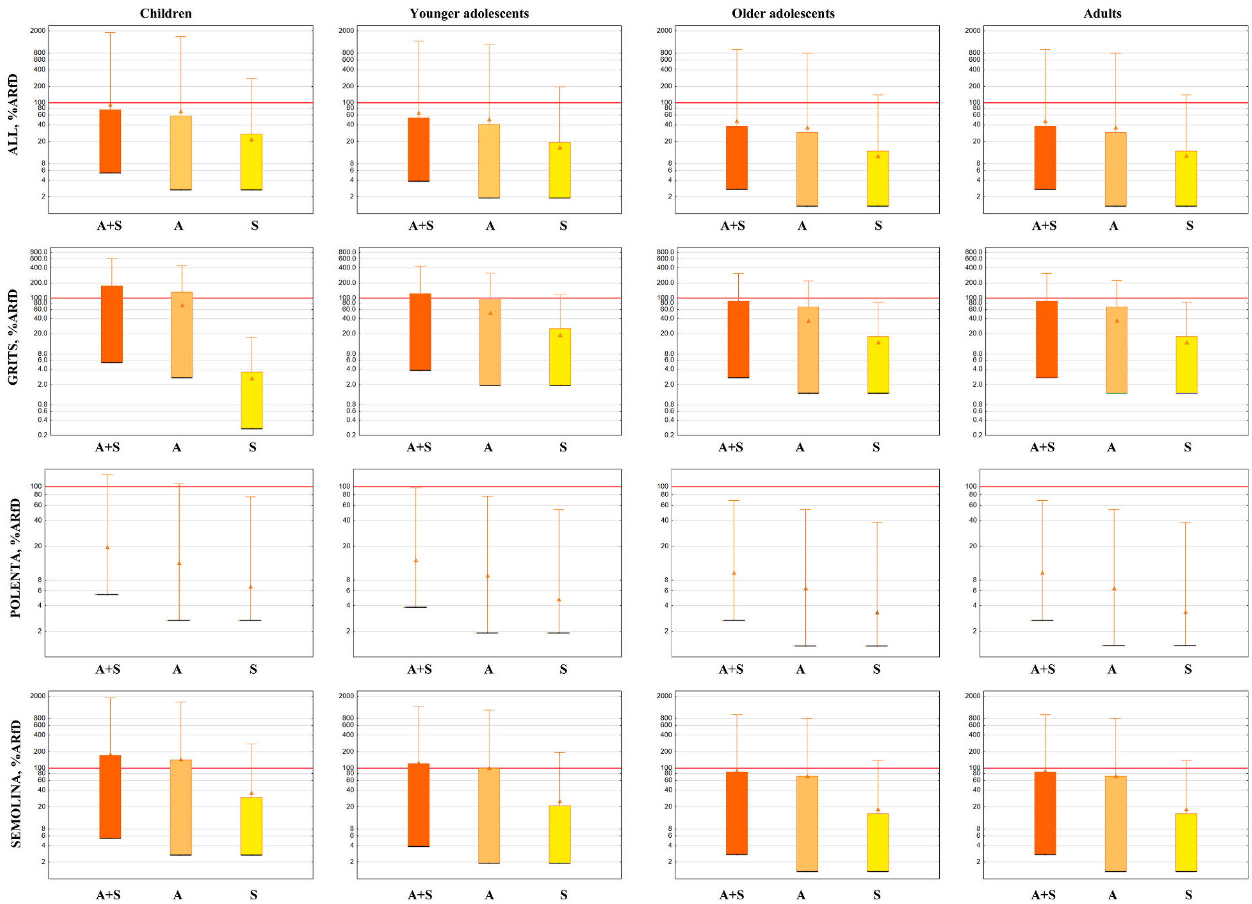

Fig. 2. Box-Whisker plot of health risk estimates associated to tropane alkaloids (sum of atropine and scopolamine (A + S), atropine (A) and scopolamine individually (S)) present in maize milling food products, expressed as % contribution to group ARfD (0.016 μg/kg bw), across age categories, under the upper bound approach. Whiskers extending from minimum to maximum, □ interquartile range, – median, Δ mean.

samples resulted in the most elevated exposures: 6.5-, 4.6-, 3.2- and 3.2-fold the group ARfD, across age categories. Importantly, all occurrence data in the last quartile were quantified values (LB=UB). The polenta group was an exception in two ways: first, 30% of the last quartile results were left-censored; second, high exposure estimates were below the group ARfD for each age category.

From a toxicological perspective, exposure exceeding the ARfD is undesirable, as adverse health effects could not be ruled out, at least not with acceptable degree of certainty. Considering the substantial probability and extent of estimated excessive exposure, consumption of the samples collected from the Serbian market would pose a health concern. However, as polenta samples contribute minimally to the group of samples exceeding the group ARfD, polenta could be the most favorable dietary option, especially for children (proportion of positive samples being 17.9%, high (UB) and maximum exposure estimates being 0.6 and 1.4-fold the group ARfD, respectively).

3.2.2. Margin of exposure

The acute dietary exposure to TAs, as evaluated in comparison with the clinically significant minimal acute effect dose (1.54 μg/kg bw for combined exposure to atropine and scopolamine), is presented in Fig. 3, for all age categories. Considering the additional sensitivity pertained due to malnutrition in the population of several African countries involved in the TA poisoning incident that prompted this assessment, both the FAO and WHO considered MOE ≥30 as acceptable [7]. In the current study, the lowest margins of exposure related to an individual product ranged from 5 to 10, across age categories, with the lowest values for children (MOE = 5). As already observed, the type of the samples being responsible for the lowest MOE was semolina, followed by grits (MOE = 16), whereas the safety profile of polenta was characterized by the most favorable MOE of 70 (all values related to children). Considering all the samples, the mean MOE for combined exposure to atropine and scopolamine ranged from 1194 to 2383, while the MOE corresponding to the P95 percentile of exposure ranged from 28 to 56, depending on the age category (the lowest values were observed in children). If compared with doses required to cause potentially adverse effects (increased heart rate, decreased saliva, pupil dilation, dry mouth and sweat secretion, etc., at 4.62 μg/kg bw; [7]), the observed acute exposure levels would lead to 3-fold greater MOE values.

The EFSA examined the differences in the approach for hazard characterization between the EFSA [9] and FAO/WHO [7], both based on the study by Perharič et al. [14]. Namely, the EFSA approach resulted in a health-based guidance value, based on the dose

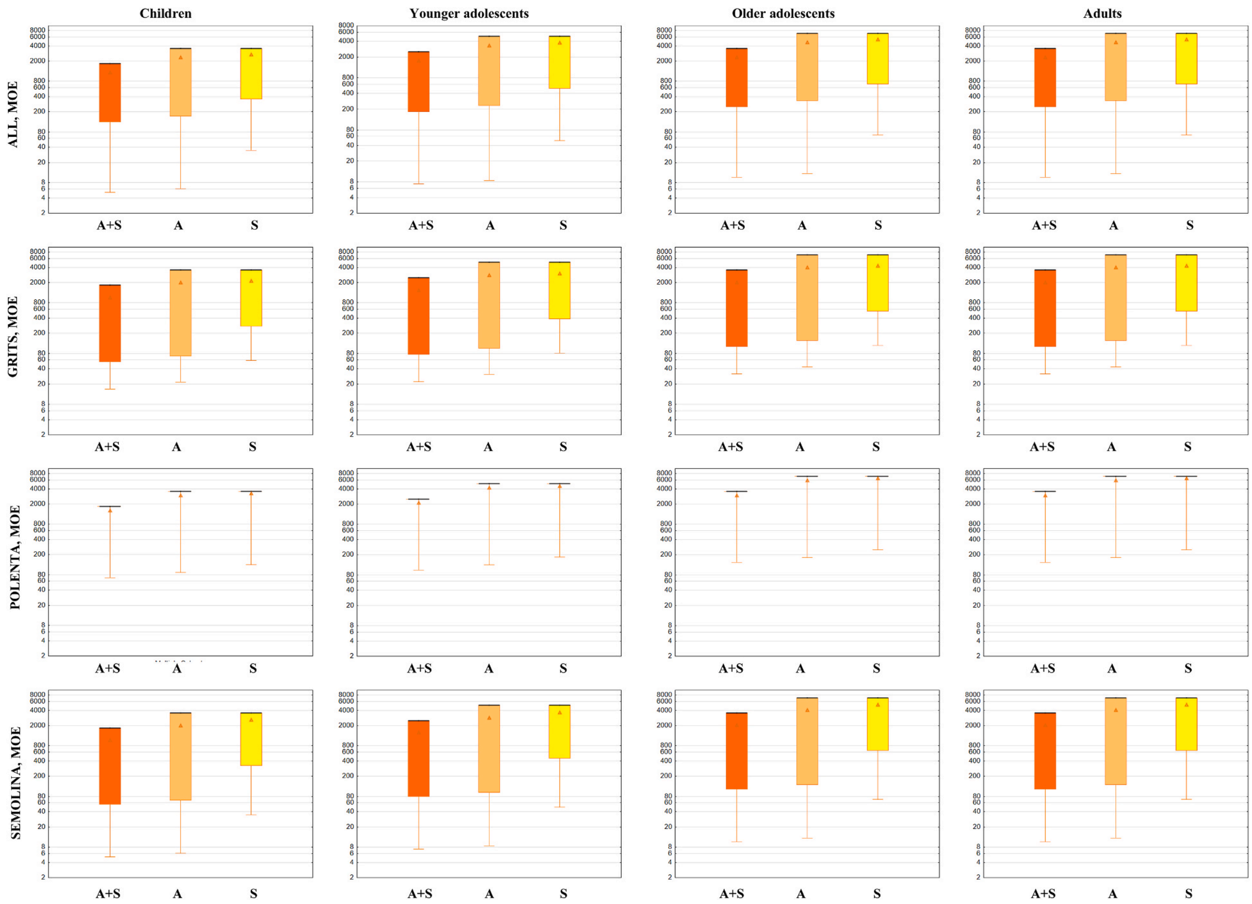

Fig. 3. Box-Whisker plot of health risk estimates associated to tropane alkaloids (sum of atropine and scopolamine (A + S), atropine (A) and scopolamine individually (S)) present in maize milling food products, expressed as an margin of exposure (based on clinically significant minimal acute dose, 1.54 μg/kg bw), across age categories, under the upper bound approach. Whiskers extending from minimum to maximum, □ interquartile range, − median, Δ mean.

responsible for a decreased heart rate, not adverse in healthy individuals, but potentially adverse in more susceptible ones. The dose selected by FAO/WHO as a point of departure for MOE calculation did not represent a level of adversity in relation to the effect on salivary secretion. Instead, it was used as a sensitive biomarker of antimuscarinic effects. However, the selected dose was within the range of the minimum therapeutic doses of (−)-hyoscyamine, atropine and (−)-scopolamine and could be associated with adverse side effects and therefore it was not applicable to more susceptible individuals with contraindications [29].

Nevertheless, the findings of the current study were not encouraging. Considering the package size (ranging from 250 to 500 g), prolonged exposure could persist over several days. However, since TAs "are not bioaccumulative, or genotoxic, and do not exhibit chronic toxicity", no tolerable semi-chronic or chronic intake have been derived [9]. The risk assessment study conducted in EU countries showed that, under the UB assumption, the group ARfD was exceeded at the mean level of exposure in toddlers and other children, and at the P95 percentile in all age groups [23]. These findings seems more serious than the ones obtained in the current Serbian study (mean exposure < ARfD in all age groups; P95 > ARfD in all age groups). However, it has to be emphasized that the EFSA study was comprehensive in its representation of a wide spectrum of food groups. Regardless, the present study clearly demonstrated that, despite its limited scope, measures to mitigate the concerns posed by TA exposure need to be considered. A previous small-scale Serbian study, conducted on maize-puffs, showed low levels of contamination and no risk of acute effects of TA in any population sub-group [30]. A recent Spanish study investigated spices and herbal infusions as potential sources of TA exposure. Estimated atropine and scopolamine exposure from fennel was 0.009 ng/kg/day, well below the ARfD. The same consideration was made for the consumption of herbal infusions, resulting in TA exposure corresponding to 3% of the ARfD or, in the case of children, less than 1%. The authors have observed that the levels of contamination found in the tested samples do not pose a health risk [31]. Dutch researchers reported that the ARfD for TAs would have been exceeded by young children when consuming 8.6% of cereal-based food for infants and young children sampled between 2011 and 2012 [27]. Several studies reported poisoning of consumers: a case study from Slovenia dealing with a mass poisoning incident caused by the consumption of buckwheat flour food products reported that the consumers were exposed to 0.7–137.6 and 0.4–63.5 μg/kg bw of atropine and scopolamine, respectively, responsible for symptoms such as dry mouth, hot red skin, blurred vision, urinary retention, tachycardia, ataxia, speech disturbance, disorientation and visual hallucinations [32]; TA contamination of cereals and soybean blend product caused poisoning events and deaths in Uganda in 2019 [13]. The warnings based on toxicological thresholds, without registered consumers' complaints, as in the current study, could not be compared with seriousness of poisoning events. However, if properly addressed, it could substantially contribute to the prevention of potential future adverse health events.

3.3. Overall considerations, including study limitations and uncertainties

The significant difference ($p < 0.05$) observed between maize milling products, grits and semolina on one hand, and polenta on the other, seems noteworthy. There is a lack of data on the effect of milling on the stability of tropane alkaloids [33], but there is a possibility of their redistribution or concentration in certain milling fractions. To mitigate the effect of heterogeneity of contamination on the variance of test results, the same principles used in mycotoxin sampling process could be applied to the analysis of TA [7].

The uncertainty of the analytical measurement is a crucial factor in the assessment of the overall uncertainty of the exposure assessment, together with uncertainties related to consumption data. Initially, while the determination of TAs was not stereo-selective, and obtained data reflected the presence of a racemic mixture of (+) and (−)-hyoscyamine (atropine) as well as (+) and (−)-scopolamine instead of biologically active (−)-enantiomers, the group ARfD was established for the sum of atropine and scopolamine, based on the fact that the biosynthesis of hyoscyamine and scopolamine in plants is directed towards the toxicologically relevant (−)-enantiomers. Therefore, the determined quantities of alkaloids could be considered as the dose that would cause a toxic biological response. Uncertainty related to other aspects of the analytical procedure was managed through method validation. In the second instance, the large proportion of left-censored results could cause substantial differences between the LB and the UB exposures and thus indicate a high level of uncertainty. However, besides formal differences associated with the distribution of left-censored results, no substantial differences were observed between the LB and UB estimates. Indeed, the highest quartile remained the same, regardless the LB/UB approach, in all groups except for polenta.

Considering that all tested maize milling products must undergo further processing to become ready for consumption, the composition and final concentration of TA could be modified. Hence, the stability of TA could be a source of substantial uncertainty in exposure studies based on semi-processed products. The EFSA has recommended stability studies, including the evaluation of potential degradation products [9]. Numerous conflicting findings has been observed in the thermal degradation studies related to cereal foods, as summarized by Kaltner [34] regarding the behavior of different classes of alkaloids, including TA, toward thermal processing or storing. On the one hand, it has been shown that: the cooking of buckwheat flour fortified with pure alkaloids resulted in losses as high as 63% of hyoscyamine and 42% of scopolamine [32]; bread-making using buckwheat and millet flour showed an overall loss of 65–70% of hyosciamine and 70–80% of scopolamine during proofing and baking [35]; during pasta cooking, TA concentration decreased due to degradation and migration to the aqueous phase; thus, the treatment with boiling water was suggested as an effective decontamination method, and regulation of TA levels in processed food was proposed based on the processing losses [36]; the baking of breadsticks prepared with TA-contaminated corn flour resulted in a decrease that was 7–65% for atropine, depending on the preparation conditions, and 35–49% for scopolamine and anisodamine [15]. On the other hand, as much as 72–100% of the TA could persist after baking bread from contaminated wheat flour [37]; during normal baking conditions for cookies, hyoscyamine and scopolamine exhibited thermal stability [38]; TA in the fortified food matrices remained stable at all storage conditions (from −80 °C to room temperature) over a 12-month period [22]. Moreover, although theoretically possible, enantiomerisation of (−)-hyoscyamine to biologically irrelevant (+)-hyoscyamine is considered unlikely under common food processing conditions [7]. Casado et al. [32] have compiled the current state of knowledge regarding the influence of food processing and culinary preparation (thermal treatment, fermentation and infusion preparation) on the content and stability of various plant alkaloids, including TAs, and concluded that further studies are needed to delve deeper into these issues. For the purpose of the current exposure assessment, in order to maximize consumers protection, it was assumed that there was no loss of TAs during further processing, *i.e.*, that these compounds are fairly stable (in line with the opinion of FAO and WHO [7]) and that food preparation and consumption behaviors are not likely to affect the contamination level.

In an exposure study, a very important aspect to consider is a possibility of aggregate exposure, resulting from the intake of a substance from various food items. In this respect, the acute nature of the adverse effects could, to some extent, limit the probability that different food items of interest would all be contaminated and then consumed within a relevant time frame. However, the potential for aggregate TAs intake from maize and other cereals such as buckwheat, soy or rice, has to be acknowledged, and therefore listed as a limitation of the current study. Another very important aspect of cereals and cereal products safety is their susceptibility to the invasion of mycotoxin producing fungi, increasingly endangering food safety in the entire Balkan region over the course of the last decade, due to significant climate changes [39]. Recent surveys confirmed the widespread presence of mycotoxins in the same types of maize milling food products marketed in Serbia, namely aflatoxins (48.2%), ochratoxin A (30.0%) [40] and fusarium toxins - zearalenone (66.1%), deoxynivalenol (42.9%) and fumonisins (96.4%) [41], with a rather high proportion of the samples showing mycotoxin co-occurrence and contamination levels above regulatory limits, but not high enough to cause acute toxicosis. The co-occurrence of contaminants could lead to exacerbated health effects and is recognized as one of the greatest challenges in risk assessments. Yet, an approach to assess combined adverse health effects of compounds belonging to different chemical classes is still in development.

Eventually, in an effort to enhance food safety, the consideration of public awareness of food-related risks is essential. In this regard, the results of the special Eurobarometer survey "*Food safety in EU*" [1] should be acknowledged. Concerning the sources of information on food risks, the majority of respondents identified television as one of their main sources (61%), followed by exchanges with family, friends, neighbors, or colleagues (44%) and internet search engines (37%). Institutional websites (e.g., public health authorities), magazines or professional journals were cited significantly less often (17, 16 and 11%, respectively). However, doctors, university scientists or publicly-funded research organization and consumer organizations are the most trusted sources of information on food risks (over 80%). Even 41% of Europeans take for granted that the food sold is safe and do not pay attention to food safety information, while 27% find it too technical and complex and 12% simply unappealing [1]. According to the above-mentioned observations, consumers trust the scientific soundness of risk assessment and, in turn, this trust should be recognized and respected by appropriate risk communication strategies. As for the institutional websites, the European Commission established the Rapid Alert System for Food and Feed (RASFF), which presents notifications related to food risks in the EU on its RASFF web-portal (https://ec.europa.eu/food/safety/rasff_en). An overview of the notifications related to TA-contaminated food revealed 58 notifications from 1994 to 2022. Even 67% of notifications released in the last ten years were related to cereals and bakery products, including one instance of maize grits originating from Serbia, flagged as a serious risk, with 4.5 and 4.3 μg/kg of atropine and scopolamine, respectively. If the population is unaware of the risk associated with the consumption of foods generally considered healthier, such as gluten-free or organic products, this might deepen health issues, particularly for sensitive individuals with contraindications and high consumers.

4. Conclusion

The levels of TA recorded in maize food products sampled on the Serbian market ranged from significantly lower to nearly 19-fold higher than the concentration that would be responsible for exceeding the group ARfD for atropine and scopolamine. Conversely, the results of the margin of exposure indicated an acceptable risk from the standpoint of clinically significant acute effects.

With regard to risk communication, if presented in an appropriate manner and format, the findings of the current study could draw the attention of not only food scientists but also the general public, particularly those concerned about contaminants in food and health-impact of food. Nevertheless, first and foremost, such findings demand attention of the food safety authority. Otherwise, not only would it be a missed opportunity to upgrade the effectiveness of the national food control system, but also none of its principal objectives would be met.

Author contribution statement

Ljilja D. Torović: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.
Vojislava Bursić: Contributed reagents, materials, analysis tools or data.
Gorica Vuković: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Data availability statement

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] EFSA (European Food Safety Authority), Special Eurobarometer Wave EB97.2 Food Safety in the EU, 2022. Retrieved on October 15, 2022 from: https://www.efsa.europa.eu/sites/default/files/2022-09/EB97.2-food-safety-in-the-EU_report.pdf.
[2] Fao and Who (Food and Agriculture Organization of the United Nations and World Health Organization), Assuring Food Safety and Quality: Guidelines for Strengthening National Food Control Systems. Food and Nutrition Paper 76, 2003. Retrieved on October 15, 2022 from, https://www.fao.org/publications/card/fr/c/92f82d38-5557-4ca1-b361-be14cd129db6/.
[3] EC (European Commission), Commission recommendation 2015/976 of 19 June 2015 on the monitoring of the presence of tropane alkaloids in food, Off. J. Eur. Union 157 (2015) 97.
[4] EC (European Commission), Commission Regulation (EU) 2016/239 of 19 February 2016 amending Regulation (EC) No 1881/2006 as regards maximum levels of tropane alkaloids in certain cereal-based foods for infants and young children, Off. J. Eur. Union L45 (2016) 3–5.
[5] EC (European Commission), Commission regulation (EU) 2021/1408 of 27 August 2021 amending Regulation (EC) No 1881/2006 as regards maximum levels of tropane alkaloids in certain foodstuffs, Off. J. Eur. Union L304 (2021) 1–4.
[6] EC (European Commission), Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs, Off. J. Eur. Union, L364 (2006) 5.
[7] FAO and WHO (Food and Agriculture Organization of the United Nations and World Health Organization). Joint FAO/WHO Expert meeting on tropane alkaloids - 30 March – 3 April 2020. Food Safety and Quality Series No. 11, 2020. Rome.Retrieved on October 15, 2022 from: https://doi.org/10.4060/cb1857en..
[8] M. de Nijs, C. Crews, F. Dorgelo, S. MacDonal, P.J.P. Mulder, Emerging issues on tropane alkaloid contamination of food in Europe, Toxins 15 (2023) e98, https://doi.org/10.3390/toxins15020098.

[9] EFSA (European Food Safety Authority), Scientific Opinion on Tropane alkaloids in food and feed, 3386, EFSA J. 11 (10) (2013) 113.
[10] M. Cirlini, T.M. Demuth, A. Biancardi, M. Rychlik, C. Dall'Asta, R. Bruni, Are tropane alkaloids present in organic foods? Detection of scopolamine and atropine in organic buckwheat (*Fagopyron esculentum* L.) products by UHPLC–MS/MS, Food Chem. 239 (2018) 141–147, https://doi.org/10.1016/j.foodchem.2017.06.028.
[11] L. González-Gómez, S. Morante-Zarcero, D. Pérez-Quintanilla, I. Sierra, Occurrence and chemistry of tropane alkaloids in foods, with a focus on sample analysis methods: a review on recent trends and technological advances, Foods 11 (2022) e407, https://doi.org/10.3390/foods11030407.
[12] T.Y.K. Chan, Worldwide occurrence and investigations of contamination of herbal medicines by tropane alkaloids, Toxins 9 (2017) e284, https://doi.org/10.3390/toxins9090284.
[13] S.A. Haughey, O.P. Chevallier, C. McVey, C.T. Elliott, Laboratory investigations into the cause of multiple serious and fatal food poisoning incidents in Uganda during 2019, Food Control 121 (2021), e107648, https://doi.org/10.1016/j.foodcont.2020.107648.
[14] L. Perharič, K.A. Juvan, L. Stanovnik, Acute effects of a low-dose atropine/scopolamine mixture as a food contaminant in human volunteers, J. Appl. Toxicol. 33 (2013) 980–990, https://doi.org/10.1002/jat.2797.
[15] F.L. Vera-Baquero, S. Morante-Zarcero, I. Sierra, Evaluation of thermal degradation of tropane and opium alkaloids in gluten-free corn breadsticks samples contaminated with Stramonium seeds and baked with poppy seeds under different conditions, Foods 11 (2022), e2196, https://doi.org/10.3390/foods11152196.
[16] T. Maslac, Serbia: Grain and Feed Annual. US Department of Agriculture (USDA): Foreign Agricultural Service, Global Agricultural Information Network. GAIN Report No RB2022-0004, 2022.
[17] G. Vuković, T. Stojanović, B. Konstantinović, V. Bursić, N. Puvača, M. Popov, N. Samardžić, A. Petrović, D. Marinković, S. Roljević Nikolić, R. Đurović Pejčev, B. Špirović Trifunović, Atropine and scopolamine in maize products from the retail stores in the Republic of Serbia, Toxins 14 (2022) 621, https://doi.org/10.3390/toxins14090621.
[18] EFSA (European Food Safety Authority), Management of left-censored data in dietary exposure assessment of chemical substances, EFSA J. 8 1557 (3) (2010) 96.
[19] Fao and Who (Food and Agriculture Organization of the United Nations and World Health Organization), Principles and Methods for the Risk Assessment of Chemicals in Food, WHO, Geneva, 2009.
[20] EFSA (European Food Safety Authority), Guidance on use of EFSA pesticide residue intake model (EFSA PRIMo revision 3), 5147, EFSA J. 16 (2018) 43.
[21] EFSA (European Food Safety Authority), Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data, 2579, EFSA J. 10 (3) (2012) 32.
[22] P.P.J. Mulder, M. de Nijs, M. Castellari, M. Hortos, S. MacDonald, C. Crews, J. Hajslova, M. Stranska, Occurrence of tropane alkaloids in food. EFSA Support, Publ 13 (2016) 1140E.
[23] EFSA (European Food Safety Authority), Human acute exposure assessment to tropane alkaloids, 5160, EFSA J. 16 (2) (2018) 24.
[24] Q. Baslé, C. Mujahid, T. Bessaire, Application of a streamlined LC-MS/MS methodology for the determination of atropine and scopolamine in cereals from Asian and African countries, Food Addit. Contam. Part A 37 (2020) 1744–1754, https://doi.org/10.1080/19440049.2020.1800828.
[25] A. Veršilovskis, P.P.J. Mulder, D.P.K.H. Pereboom-de Fauw, J. de Stoppelaar, M. de Nijs, Simultaneous quantification of ergot and tropane alkaloids in bread in The Netherlands by LC-MS_MS, Food Addit. Contam. Part B Surveill. 13 (2020) 215–223, https://doi.org/10.1080/19393210.2020.1771777.
[26] H. Chen, J. Marín-Sáez, R. Romero-González, A. Garrido Frenich, Simultaneous determination of atropine and scopolamine in buckwheat and related products using modified QuEChERS and liquid chromatography tandem mass spectrometry, Food Chem. 218 (2017) 173–180, https://doi.org/10.1016/j.foodchem.2016.09.075.
[27] P.P.J. Mulder, D.P.K.H. Pereboom-de Fauw, R.L.A.P. Hoogenboom, J. de Stoppelaar, M. de Nijs, Tropane and ergot alkaloids in grain-based products for infants and young children in The Netherlands in 2011–2014, Food Addit. Contam. Part B Surveill. 8 (2015) 284–290, https://doi.org/10.1080/19393210.2015.1089947.
[28] J. Marín-Sáez, R. Romero-González, A.G. Garrido Frenich, Reliable determination of tropane alkaloids in cereal based baby foods coupling on-line SPE to mass spectrometry avoiding chromatographic step, Food Chem. 275 (2019) 746–753, https://doi.org/10.1016/j.foodchem.2018.09.137.
[29] EFSA (European Food Safety Authority), Scientific report on the assessment of the conclusions of the joint FAO/WHO Expert meeting on tropane alkaloids, 7229, EFSA J. 20 (4) (2022) 17, https://doi.org/10.2903/j.efsa.2022.7229.
[30] T. Stojanović, G. Vuković, A. Petrović, B. Konstantinović, N. Puvača, D. Marinković, S. Gvozdenac, V. Bursić, Determination of tropane alkaloids in corn puffs by the LC-MS/MS, Matica Srpska J. Nat. Sci. 141 (2021) 69–80, https://doi.org/10.2298/ZMSPN2141069S.
[31] L. González-Gómez, J. Gañán, S. Morante-Zarcero, D. Pérez-Quintanilla, I. Sierra, Atropine and scopolamine occurrence in spices and fennel infusions, Food Control 146 (2023), 109555, https://doi.org/10.1016/j.foodcont.2022.109555.
[32] L. Perharič, G. Kozelj, B. Druzina, L. Stanovnik, Risk assessment of buckwheat flour contaminated by thorn-apple (*Datura stramonium* L.) alkaloids: a case study from Slovenia, Food Addit. Contam. Part A. 30 (2013) 321–330, https://doi.org/10.1080/19440049.2012.743189.
[33] N. Casado, G. Casado-Hidalgo, L. González-Gómez, S. Morante-Zarcero, I. Sierra, Insight into the impact of food processing and culinary preparations on the stability and content of plant alkaloids considered as natural food contaminants, Appl. Sci. 13 (2023), e1704, https://doi.org/10.3390/app13031704.
[34] F. Kaltner, Fate of food-relevant toxic plant alkaloids during food processing or storing and analytical strategies to unveil potential transformation products, J. Agric. Food Chem. 70 (2022) 5975–5981, https://doi.org/10.1021/acs.jafc.2c01489.
[35] J. Marín-Sáez, R. Romero, A.G. Garrido Frenich, Degradation of tropane alkaloids in baked bread samples contaminated with Solanaceae seeds, Food Res. Int. 122 (2019) 585–592, https://doi.org/10.1016/j.foodres.2019.01.027.
[36] J. Marín-Sáez, R. Romero-González, A.G. Garrido Frenich, Effect of tea making and boiling processes on the degradation of tropane alkaloids in tea and pasta samples contaminated with Solanaceae seeds and coca leaf, Food Chem. 287 (2019) 265–272, https://doi.org/10.1016/j.foodchem.2019.02.091.
[37] M. Friedman, C.E. Levin, Composition of jimson weed (*Datura stramonium*) seeds, J. Agric. Food Chem. 37 (1989) 998–1005, https://doi.org/10.1021/jf00088a040.
[38] B. Tong, M. Schild, R. Bickel, H. D'ierauer, Tropanalkaloide in Biolebensmitteln. Forschungsinstitut für biologischen Landbay FiBL, Schlussbericht. Switzerland, 2017, p. 30.
[39] Lj Torović, N. Popov, M. Živkov-Baloš, S. Jakšić, Risk estimates of hepatocellular carcinoma in Vojvodina (Serbia) related to AFM1 contaminated cheese, J. Food Compos. Anal. 103 (2021), e104122, https://doi.org/10.1016/j.jfca.2021.104122.
[40] Lj Torović, Aflatoxins and ochratoxin A in flour: a survey of the Serbian retail market, Food Addit. Contam. Part B Surveill. 11 (1) (2018) 26–32, https://doi.org/10.1080/19393210.2017.1391335.
[41] Lj Torović, *Fusarium* toxins in corn food products: a survey of the Serbian retail market, Food Addit. Contam. Part A. 35 (8) (2018) 1596–1609, https://doi.org/10.1080/19440049.2017.1419581.