Chemistry and Occurrence of Tropane Alkaloids in Foods

Subjects: Chemistry, Analytical

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Tropane alkaloids (TAs) are natural toxins produced by different plants, mainly from the Solanaceae family.

tropane alkaloids food atropine scopolamine food sample preparation

1. Introduction

Tropane alkaloids (TAs) are secondary metabolites produced by various plant species, mainly belonging to the Solanaceae family, in addition to a variety of other families (Brassicaceae, Erythroxylaceae, Euphorbiaceae, Convulvulaceae) [1][2][3]. There are more than 200 different TAs identified, and they can be found anywhere in the plant, including seeds, fruits, flowers, leaves and stems [2][4]. The most studied TAs in foods are atropine (At) and scopolamine (Sc).

Crop contamination by plants of the Solanaceae family is the most widespread source of accidental TAs consumption. Specifically, *Datura*, *Hyoscyamus* and *Atropa* species are the main ones responsible for food and feed products contamination by TAs. This is because they grow easily as weeds in crops of different plant-foods. In addition, these alkaloids are found in all parts of TA-producing plants, so cross-contamination especially with seeds but also with leaves, roots, fruits and flowers is frequent due to fast and mechanical harvesting. The seeds of *Datura stramonium* (jimson weed or thorn apple), widely distributed in all the warm regions of the world, along with other *Datura* spp., such as *D. ferox* are the ones that appear the most in foods [3]. *D. stramonium* produces numerous seeds that are encapsulated in a kind of apple-shaped fruit, hence its name thorn apple. These seeds have generally been found as impurities in numerous crops of linseed, soy, millet, sunflower and buckwheat [5] (**Figure 1**). For example, in Uganda in 2019, humanitarian food aid (product known as Super Cereal composed by maize and soya) contaminated with TAs of *D. stramonium* was responsible of the foodborne outbreak which caused over 300 hospitalizations and 5 deaths [6]. In this sense, it is necessary that food producers and manufacturing companies ensure, in the next years, the reduction of the amount of undesirable plants producing TAs in crops, raw materials and finished products, following good agricultural and manufacturing practices. In addition, the adequate application of food safety control measures (including the establishment of maximum limits) can aid in the reduction of TAs in foods [6].

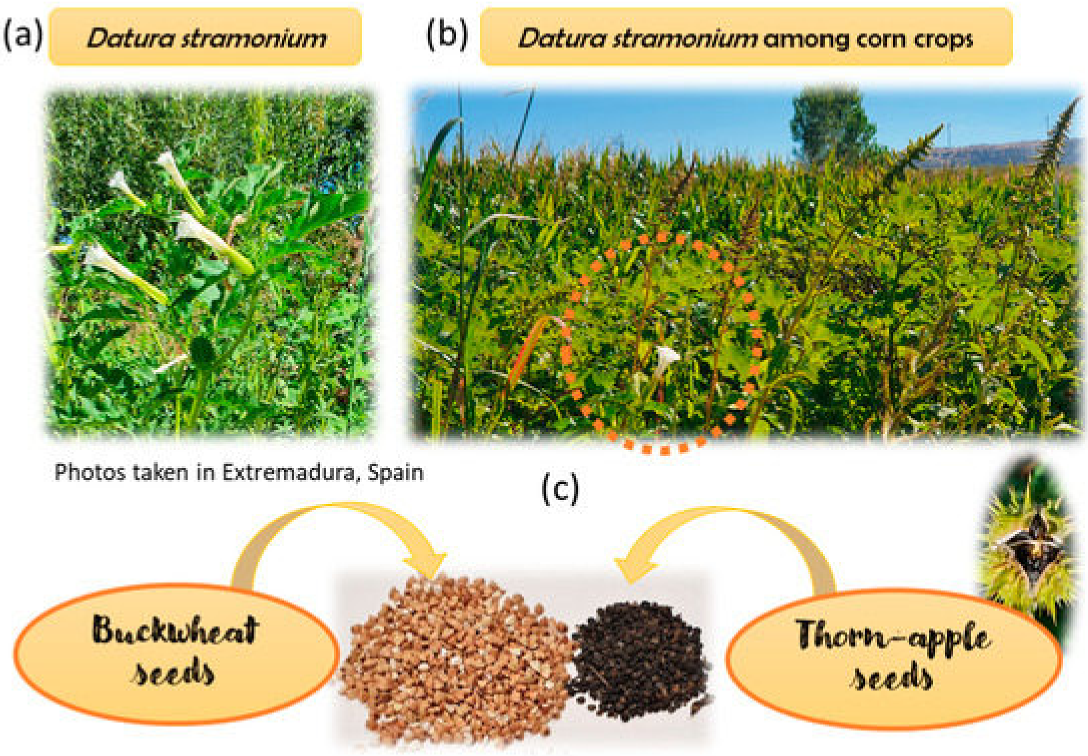

Figure 1. (**a**) *Datura stramonium* plant, (**b**) *Datura stramonium* among corn crops and (**c**) similarity of *Datura stramonium* seeds with buckwheat seeds.

2. Chemistry and Occurrence of TAs in Foods

2.1. Chemistry

TAs are a type of alkaloids with a tropane ring in their chemical structure, characterized by a two-ringed structure with pyrrolidine and piperidine rings sharing a single nitrogen atom and two carbon atoms (**Figure 2**). Most of them are esters derived from organic acids and hydroxytropanes [2]. The most important TAs in foods are At, which is a racemic mixture of (±)-hyoscyamine, and (−)-Sc or hyoscine. Although cocaine and calystegines have also a tropane ring (**Figure 2**), so they can be included in the TAs group, these compounds will not be discussed in this review. Cocaine and other coca alkaloids from *Erythoxylum coca* have no interest in food analysis, as they are used as a drug of abuse. Calystegines are recently discovered polyhydroxylated nortropane alkaloids. Due to their inability to pass the blood–brain barrier, these compounds do not show any psychoactive effects and, until now, their occurrence and analysis in food samples have received little research attention [7].

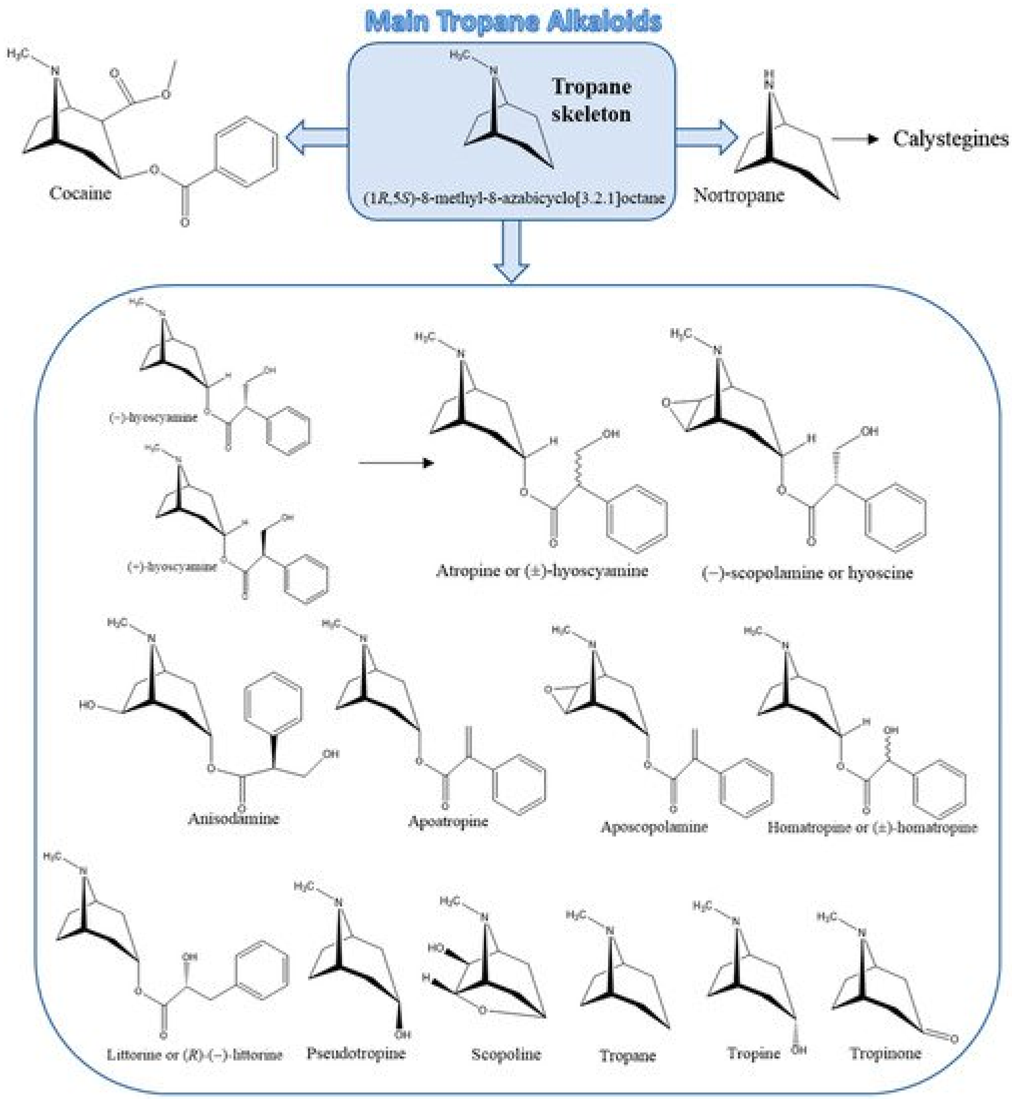

Figure 2. Common skeleton structure of tropane and principal tropane alkaloids.

Despite all TAs having the same basic structure, they differ in their biological and pharmacological properties. At and Sc have been used throughout history in medicine, generally administered at low doses as drugs in the form of salts, such as atropine sulfate, or as semi-synthetic derivatives such as homatropine bromide or N-butylscopolamine bromide [1]. Their use has served to treat multiple symptoms and pathologies such as nausea, vomiting, heart or respiratory problems and also as an antispasmodic in gastrointestinal problems, as antiallergic drugs, as a treatment against organophosphate compounds and even as a pupil dilator for ophthalmic treatment [7][8][9][10]. However, and despite their great use, these compounds have been implicated in numerous intoxications due to the consumption of TA-containing plants or foods contaminated with these plants. Its toxic effects are due to the fact that they are anticholinergic compounds, which avoid the binding of acetylcholine with the muscarine receptor [1][4][8][11]. These toxic effects cause tachycardia, muscle spasms, mydriasis, delirium and sometimes can even cause death [2][8].

2.2. Occurrence

2.2.1. Cereals, Pseudo-Cereals, Legumes and Grains

Contaminated cereals, pseudo-cereals, legumes and grains are of particular importance because these kinds of food-products are a major part of the diet for all age groups in the population. In relation to TAs' occurrence, one of the most studied has been buckwheat (*Fagopyron esculentum* L.). In fact, this pseudo-cereal has caused great commercial interest for its healthy properties, being rich in polyphenols, vitamins and proteins [12]. Buckwheat flour and other gluten-free flours (i.e., amaranth, chickpea, pea, corn, rice, millet and quinoa flours, among others) are used as wheat flour substitutes in the production of gluten-free bread, cakes, cookies, pasta and snacks. These gluten-free foods have gained great popularity in the last years, not only in the celiac community, which emphasizes the need to carefully control the occurrence of TAs present in these products. This is of especial importance as the seeds of *Datura stramonium* are very similar in size and shape to those of buckwheat and other seed matrices such as linseed, soy, sorghum, and millet (**Figure 1**c). Additionally, the commercialization of organic foods is common so that the use of phytosanitary products is avoided, which results in the crops growing with a greater number of weeds. This can cause the contamination of the flours and derived products such as pasta, bread or baby foods, which are currently highly consumed.

Table 1 shows the occurrence of TAs in cereals, pseudo-cereals, legumes and grains. In some studies, high concentrations of At and Sc have been found, together with other alkaloids. For example, in the study of 26 buckwheat-derived organic foods carried out by Cirlini et al. [13], At and Sc were found in a sample of buckwheat flour (83.9 and 10.4 µg/kg, respectively) and in a sample of buckwheat pasta (21.3 and 5.7 µg/kg, respectively). In addition, positive samples in At were found by González-Gómez et al. [14] in buckwheat grains and flours in a range between 6.7 and 21 µg /kg. Other cereals, pseudo-cereals, legumes and grains are comparatively less studied. However, amounts that exceed the maximum level allowed for millet and sorghum according to the Commission Regulation (EU) 2021/1408 [15] have been reported. In that respect, Marín et al. [16] found 13 µg/kg of At and 23 µg/kg of Sc in a sample of millet flour, and in the study of González-Gómez et al. [14], a sorghum flour contained 15 µg/kg. In the same study, in a sample of teff flour, the sum of At and Sc was higher than 100 µg/kg, which evidenced that these kinds of grains and flours must also be carefully studied.

Table 1. Occurrence of TAs in cereals, pseudo-cereals, legumes and grains.

Foods (Nº of Samples Analyzed)	Nº of Samples with At (Range)	Nº of Samples with Sc (Range)	Other TAs	[Ref.]
Breakfast cereals, breakfast cereals with milk, biscuits, cookies (113 samples)	21 of breakfast cereals (0.09–65.6 µg/kg)	18 of breakfast cereals (0.28–15.2 µg/kg)	Anisodamine, aposcopolamine, homatropine, anidosine	[17]
Flours (buckwheat, millet and corn), cereal-based foods for children, breakfast cereals, biscuits	46 of flours (0.5–149 µg/kg), 42 of cereal-based foods for children (0.5–3.73 µg/kg),	46 of flours (0.5–198.5 µg/kg), 42 of cereal-based foods for children (0.5–1.86 µg/kg), 15 of	6-HO-tropinone, nortropinone, pseudotropine, scopine, scopoline, tropine, tropinone,	[18]

Foods (Nº of Samples Analyzed)	Nº of Samples with At (Range)	Nº of Samples with Sc (Range)	Other TAs	[Ref.]
and pastry, pasta and bread, legumes, stir-fry mixes and oilseeds (1305 samples)	15 of breakfast cereals (0.5–90.83 µg/kg), 24 of biscuits and pastry (0.5–1.85 µg/kg), 18 of bread (0.5–3.80 µg/kg), 20 of legumes, stir-fry mixes and oilseeds (0.5–0.11 µg/kg)	breakfast cereals (0.5–17.64 µg/kg), 24 of biscuits and pastry (0.5–0.65 µg/kg), 18 of bread (0.5–0.36 µg/kg), 20 of legumes, stir-fry mixes and oilseeds (0.5–0.09 µg/kg)	convolamine, convolidine, convolvine, fillalbine, anisodamine, apoatropine, aposcopolamine, homatropine, littorine, noratropine, norscopolamine	
Buckwheat, buckwheat flour and pasta; soy and soy flour; peeled millet and millet flour; linseed and linseed flour (15 samples)	1 of buckwheat (<1 µg/kg) 1 of millet flour (13 µg/kg)	1 of buckwheat (<2 µg/kg) 1 of millet flour (23 µg/kg)	Anidosamine, littorine, tropinone	[16]
Buckwheat, buckwheat flour and pasta, soy, wheat, amaranthus grain, chia seeds, peeled millet (8 samples)	N.D	N.D	Three scopolamine transformation products	[19]
Buckwheat flour, pasta and bakery (26 samples)	1 of flour (83.9 µg/kg) 1 of pasta (21.3 µg/kg) 1 of bakery (13.9 µg/kg)	1 of flour (10.4 µg/kg) 1 of pasta (5.7 µg/kg)	N.St	[13]
Cereal based baby foods (pap, biscuits, snacks and grissines) (18 samples)	1 of biscuits (11.5 µg/kg)	1 of biscuits (2.8 µg/kg)	Anidosamine, homatropine, apoatropina	[20]
Bread (wheat, multi-grain, rye, wheat-rye) (40 samples)	N.D	1 of wheat-rye bread (0.22 µg/kg)	N.D	[21]
Wheat, corn, rice, oat and millet flours, mixed cereals flours, infant cereals, cereal-based products (95 samples)	1 of tomato rice flakes product (9.6 µg/kg)	1 of tomato rice flakes product (2.6 µg/kg)	N.St	[22]

Foods (Nº of Samples Analyzed)	Nº of Samples with At (Range)	Nº of Samples with Sc (Range)	Other TAs	[Ref.]
Buckwheat and buckwheat flour, quinoa, amaranth, teff flour, refined corn flour, corn flour, blue corn flour, sorghum flour, peeled millet, green and red lentil flours, chickpea flour, pea flour (15 samples)	3 of buckwheat grain and flour (6.7–21 µg/kg), 1 of quinoa (7.1 µg/kg), 1 of teff flour (78 µg/kg), 1 of refined corn flour (7 µg/kg), 1 of sorghum flour (15 µg/kg), 1 of peeled millet (6.9 µg/kg)	1 of teff flour (28 µg/kg)	N.St	[14]

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TAs can pose a serious health problem, as these toxins are relatively heat-stable, and some heavily contaminated products have been found in the market, so they can contribute significantly to the population's exposure to TAs. In this sense, different poisoning cases caused by the contamination of herbal teas have been reported [23], and RASFF notifications from 2015 showed that seven alerts were found to be in teas and herbal teas (from 26.8 to 543.1 µg/kg of At and from 23 to 488.7 µg/kg of Sc). On the other hand, the current RASFF notification has evidenced that other botanical ingredients may also contain high amounts of these natural toxins. In this sense, an RASFF alert in 2018 deserves to be mentioned, due to the high TAs concentration found in whole cumin seeds from Hungary with 16178 µg/kg for At and 4658 µg/kg for Sc. The positive findings of TAs in these products warrant a more extensive survey to determine the extent of TAs contamination in this type of product.

The occurrence of TAs has been mainly studied in tea (black, green, red) but also in other herbal teas such as peppermint, chamomile and rooibos, among others [24][25][26]. In some of these products, positive samples exceeded the 25 µg/kg of TAs (as the sum of At and Sc) established as maximum level by the Commission Regulation (EU) 2021/1408 in dried products for herbal infusions (50 µg/kg in anise seeds) [15]. At and Sc were the most prominent TAs found, but others such as homatropine, anidosamine, apoatropine, physoperuvine, pseudotropine and tropine were also detected [24][25].

2.2.3. Other Plant-Based Foods

Vegetables and other plant-based food products can also be contaminated with TAs. This is because the leaves of these vegetables, for example, spinach, can be mixed with leaves from TA-producing plants such as *Datura* spp. In addition, despite boiling being a common cooking option for these products, boiling water may not be able to effectively extract TAs.

2.2.4. Animal-Derived Foods

Very little work has been carried out examining the potential presence of TAs in animal-derived foods. In 2013, the EFSA concluded that the risk of TAs poisoning through the consumption of food of animal origin was unlikely [2]. This is because cattle generally do not consume plants that contain TAs, since they tend to have an unpleasant taste that makes animals avoid it. Only when the amount of available grass is restricted are cattle likely to consume these plants [3]. Therefore, exposure to these toxins is usually mainly through feed contaminated with TAs or through waters contaminated with TA drugs that end up in rivers and are consumed by animals [27]. In a recent study by Lamp et al. [28], the transfer of TAs from feed to milk was demonstrated in cattle for the first time. These authors conclude that since the mixing of various raw milk leads to a dilution of possible TAs, the occurrence of dairy products contaminated in the market can most probably be disregarded, but the situation might be different when raw milk is sold on the farm. In this regard, the analysis of TAs content in milk and other animal-derived foods (such as meat, eggs), other than feed, could help estimate current TAs exposure levels through the food of animal origin.

References

1. Aehle, E.; Dräger, B. Tropane alkaloid analysis by chromatographic and electrophoretic techniques: An update. J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 2010, 878, 1391–1406.
2. European Food Safety Authority. Scientific Opinion on Tropane alkaloids in food and feed. EFSA J. 2013, 11, 1–113.
3. Alexander, J.; Benford, D.; Cockburn, A.; Cravedi, J.; Dogliotti, E.; Domenico, A.D.I.; Férnandez-Cruz, M.L.; Fürst, P.; Fink-gremmels, J.; Galli, C.L.; et al. Tropane alkaloids (from Datura sp.) as undesirable substances in animal feed—Scientific Opinion of the Panel on Contaminants in the Food Chain. EFSA J. 2008, 6, 1–55.
4. Kohnen-Johannsen, K.L.; Kayser, O. Tropane alkaloids: Chemistry, pharmacology, biosynthesis and production. Molecules 2019, 24, 796.
5. Martinello, M.; Borin, A.; Stella, R.; Bovo, D.; Biancotto, G.; Gallina, A.; Mutinelli, F. Development and validation of a QuEChERS method coupled to liquid chromatography and high resolution mass spectrometry to determine pyrrolizidine and tropane alkaloids in honey. Food Chem. 2017, 234, 295–302.
6. Abia, W.A.; Montgomery, H.; Nugent, A.P.; Elliott, C.T. Tropane alkaloid contamination of agricultural commodities and food products in relation to consumer health: Learnings from the 2019 Uganda food aid outbreak. Compr. Rev. Food Sci. Food Saf. 2021, 20, 501–525.
7. Romera-Torres, A.; Romero-González, R.; Martínez Vidal, J.L.; Garrido Frenich, A. Analytical methods, occurrence and trends of tropane alkaloids and calystegines: An update. J. Chromatogr. A 2018, 1564, 1–15.
8. Adamse, P.; van Egmond, H.P.; Noordam, M.Y.; Mulder, P.P.J.; De Nijs, M. Tropane alkaloids in food: Poisoning incidents. Qual. Assur. Saf. Crop. Foods 2014, 6, 15–24.
9. Dey, P.; Kundu, A.; Kumar, A.; Gupta, M.; Lee, B.M.; Bhakta, T.; Dash, S.; Kim, H.S. Analysis of Alkaloids (Indole Alkaloids, Isoquinoline Alkaloids, Tropane Alkaloids); Elsevier: Amsterdam, The

Netherlands, 2020; ISBN 9780128164556.

10. Wink, M. Alkaloids: Toxicology and Health Effects, 1st ed.; Elsevier Ltd.: Amsterdam, The Netherlands, 2015; ISBN 9780123849533.
11. Debnath, B.; Singh, W.S.; Das, M.; Goswami, S.; Singh, M.K.; Maiti, D.; Manna, K. Role of plant alkaloids on human health: A review of biological activities. Mater. Today Chem. 2018, 9, 56–72.
12. Giménez-Bastida, J.A.; Zieliński, H. Buckwheat as a Functional Food and Its Effects on Health. J. Agric. Food Chem. 2015, 63, 7896–7913.
13. Cirlini, M.; Demuth, T.M.; Biancardi, A.; Rychlik, M.; Dall'Asta, C.; Bruni, R. 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. 2018, 239, 141–147.
14. González-Gómez, L.; Gañán, J.; Morante-Zarcero, S.; Pérez-Quintanilla, D.; Sierra, I. Sulfonic Acid-Functionalized SBA-15 as Strong Cation-Exchange Sorbent for Solid-Phase Extraction of Atropine and Scopolamine in Gluten-Free Grains and Flours. Foods 2020, 9, 1854.
15. European Union. 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 2021, L304, 1–4.
16. Marín-Sáez, J.; Romero-González, R.; Garrido Frenich, A. Multi-analysis determination of tropane alkaloids in cereals and solanaceaes seeds by liquid chromatography coupled to single stage Exactive-Orbitrap. J. Chromatogr. A 2017, 1518, 46–58.
17. Mulder, P.P.J.; Pereboom-de Fauw, D.P.K.H.; Hoogenboom, R.L.A.P.; de Stoppelaar, J.; de Nijs, M. 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. 2015, 8, 284–290.
18. Mulder, P.P.J.; de Nijs, M.; Castellari, M.; Hortos, M.; MacDonald, S.; Crews, C.; Hajslova, J.; Stranska, M. Occurrence of tropane alkaloids in food. EFSA Support. Publ. 2016, 13, 1140E.
19. Chen, H.; Marín-Sáez, J.; Romero-González, R.; Garrido Frenich, A. Simultaneous determination of atropine and scopolamine in buckwheat and related products using modified QuEChERS and liquid chromatography tandem mass spectrometry. Food Chem. 2017, 218, 173–180.
20. Marín-Sáez, J.; Romero-González, R.; Garrido Frenich, A. Reliable determination of tropane alkaloids in cereal based baby foods coupling on-line spe to mass spectrometry avoiding chromatographic step. Food Chem. 2019, 275, 746–753.
21. Veršilovski, A.; Mulder, P.P.J.; Pereboom-de Fauw, D.P.K.H.; de Stoppelaar, J.; de Nijs, M. Simultaneous quantification of ergot and tropane alkaloids in bread in the Netherlands by LC-MS_MS.pdf. Food Addit. Contam. Part B 2020, 13, 215–223.

22. Baslé, Q.; Mujahid, C.; Bessaire, T. 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 Chem. Anal. Control. Expo. Risk Assess. 2020, 37, 1744–1754.

23. Chan, T.Y.K. Worldwide occurrence and investigations of contamination of herbal medicines by Tropane Alkaloids. Toxins 2017, 9, 284.

24. Cirlini, M.; Cappucci, V.; Galaverna, G.; Dall'Asta, C.; Bruni, R. A sensitive UHPLC-ESI-MS/MS method for the determination of tropane alkaloids in herbal teas and extracts. Food Control 2019, 105, 285–291.

25. Romera-Torres, A.; Romero-González, R.; Martínez Vidal, J.L.; Garrido Frenich, A. Simultaneous analysis of tropane alkaloids in teas and herbal teas by liquid chromatography coupled to high-resolution mass spectrometry (Orbitrap). J. Sep. Sci. 2018, 41, 1938–1946.

26. Shimshoni, J.A.; Duebecke, A.; Mulder, P.P.J.; Cuneah, O.; Barel, S. Pyrrolizidine and tropane alkaloids in teas and the herbal teas peppermint, rooibos and chamomile in the Israeli market. Food Addit. Contam. Part A Chem. Anal. Control. Expo. Risk Assess. 2015, 32, 2058–2067.

27. Zheng, W.; Yoo, K.H.; Choi, J.M.; Park, D.H.; Kim, S.K.; Kang, Y.S.; Abd El-Aty, A.M.; Hacımüftüoğlu, A.; Jeong, J.H.; Bekhit, A.E.D.; et al. A modified QuEChERS method coupled with liquid chromatography-tandem mass spectrometry for the simultaneous detection and quantification of scopolamine, L-hyoscyamine, and sparteine residues in animal-derived food products. J. Adv. Res. 2019, 15, 95–102.

28. Lamp, J.; Knappstein, K.; Walte, H.G.; Krause, T.; Steinberg, P.; Schwake-Anduschus, C. Transfer of tropane alkaloids (atropine and scopolamine) into the milk of subclinically exposed dairy cows. Food Control 2021, 126, 108056.