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Recent food alerts and analytical advances related to the contamination of tropane and pyrrolizidine alkaloids in food

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The food control of tropane and pyrrolizidine alkaloids has become in recent years in Europe one of the main priorities within the food safety field due to the exponential increase in food alerts related to the concerning levels of these natural toxins in foods. This awareness has led to increasing progress in the research and determination of these compounds. Hence, this mini-review provides a brief and comprehensive overview of the evolution of these food alerts, highlighting the main food products frequently contaminated with these alkaloids and reviewing the analytical advances in their determination within the las 3 years (from 2020 to 2023).

KEYWORDS

natural toxins, alkaloids, food safety, RASFF, food analysis, sample preparation, chromatographic analysis, mass spectrometry

1 Introduction

Countless plants belonging to different genera naturally present many phytochemical substances in their composition. Among these substances, the presence of alkaloids can be highlighted. Alkaloids are nitrogenous compounds synthesized as secondary metabolites that act as a defense mechanism of many plants. It is well known that these alkaloids have great pharmacological potential, and therefore their toxicity and applicability in this field have been largely studied (Thawabteh et al., 2019). However, the intake of these alkaloids can have serious health consequences (Casado et al., 2024a). Hence, awareness of the emergence of these compounds as food contaminants has increased significantly in recent years. Particularly, many of the food alerts recently notified in Europe have been related to levels of tropane and pyrrolizidine alkaloids (hereinafter TAs and PAs, respectively) (Figures 1A, B) found above legislated limits in a wide variety of food products (Casado et al., 2022a; González-Gómez et al., 2022a). Hence, within the last 3 years, increasing progress in the research of these compounds as food contaminants has been made. Consequently, the first European regulation to monitor the occurrence of these compounds in food was published in 2020 and 2021 for PAS and TAs, respectively (European Union, 2021; European Union, 2023). Nonetheless, in April 2023, these regulations were revised, updated, and extended for more food products, establishing the regulation currently in force for the control of these contaminants (European Union, 2024). Likewise, recently, a new regulation establishing the methods of sampling and

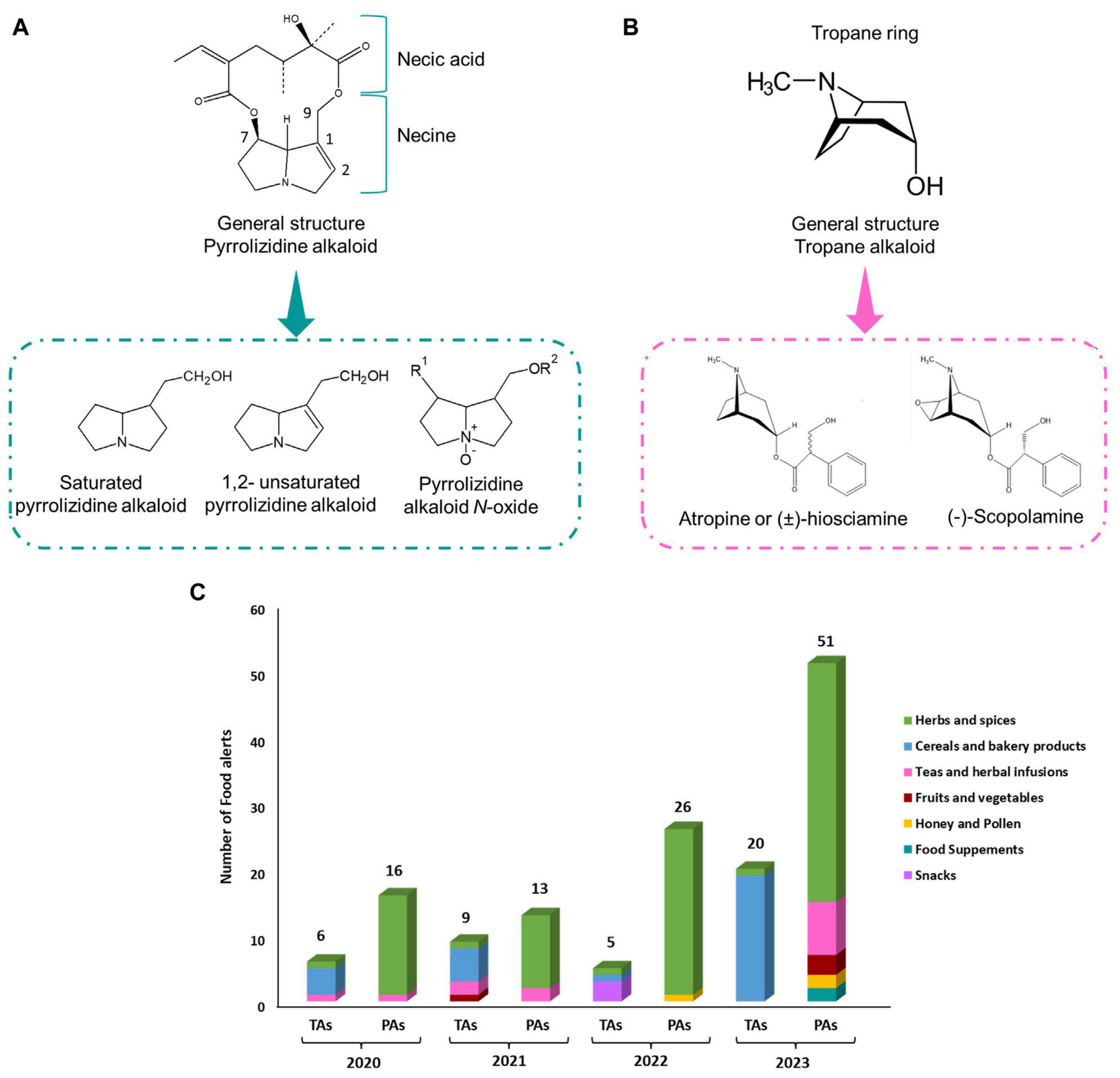

FIGURE 1
(A) General chemical structure of pyrrolizidine alkaloids (PAs) including different forms of them (R1 and R2 correspond to different necic acids), **(B)** general chemical structure of tropane alkaloids (TAs) and the main representative TAs (atropine and scopolamine), and **(C)** evolution of number of food alerts notified in the European Rapid Alert System for Food and Feed (RASFF) window related to the occurrence of TAs and PAs in different food categories from 2020 to 2023. Data obtained from the RASFF window portal from 1st January 2020 to 30th November 2023.

analysis for the control of the levels of plant toxins in food has been published (Commission implementing regulation, 2024).

Accordingly, the aim of this mini-review is to provide an overview of the evolution of the food alerts related to the occurrence of TAs and PAs in Europe within the last 3 years (from 2020 to 2023), highlighting the main food products frequently contaminated with these alkaloids and allowing to get up-to-date on the analytical methodologies developed for their determination in these food matrices within this period. Special emphasis will made on those recent strategies that allow the simultaneous determination of these two types of alkaloids, as well as on those works that have developed novel and sustainable microextraction procedures as emergent approaches in this field. Likewise, challenges and expected future trends are also included. Overall, this review intents to succinctly highlight the importance of these current food safety issue.

2 Evolution of food alerts in the last 3 years (2020–2023) and analytical methods related to TAs and PAs

Figure 1C shows the evolution of number of food alerts notified in the EU related to the occurrence of TAs and PAs in food products from 2020 to 2023, including the type of product in which these toxins have been detected. It is observed food alerts for PAs are greater than for TAs. Regulation for PAs in Europe started before than that of TAs, so it is possible there has been greater control of these alkaloids in recent years. Moreover, the first regulation for TAs contemplated less variety of products than the current one. Nonetheless, the number of alerts has increased significantly for both toxins in the last year 2023. This may be due to the entry into force of the new EU regulation for these contaminants, requiring more surveillance in a wider variety of foodstuffs (European Union, 2024).

As shown in Figure 1C, the occurrence of these toxins at high levels has primarily been notified for plant-derived products (except honey), since plants are the main source of these alkaloids. Among these plant-derived products, herbs and spices are the products with the highest degree of contamination of both toxins, followed by teas and herbal infusions. Alerts in cereals and bakery products, as well as snacks, have only been reported for the presence of TAs, while alerts in food supplements, honey and pollen exclusively correspond to PAs. In the case of fruits and vegetables, one food alert corresponding to the presence of TAs was reported in 2021, however, in 2023, several alerts have been notified due to the presence of PAs in these products. The main analytical advances carried out in these food categories for the determination of these alkaloids within the period 2020–2023 are reviewed below. It is worth mentioning that liquid chromatography (LC) or its improved variant ultra-high-performance LC (UHPLC) coupled to mass spectrometry (MS) have been mainly used for the analysis using C18 separation columns and electrospray ionization (ESI) in positive ion mode.

2.1 Determination in herbs and spices

Among this food category, food alerts notified in the last years are predominantly linked to high levels of PAs found in oregano and cumin. In contrast, the occurrence of TAs has been basically notified in savory, parsley and peppermint (Rapid Alert System for Food and Feed, 2023). The main contamination pathway of these products with these two alkaloids is during cultivation and harvesting. Frequently, many PAs- and TAs-producing plants grow as weeds among crop fields, so cross-contamination can occur (Casado et al., 2022a; González-Gómez et al., 2022a; Casado et al., 2024a). Likewise, different studies have demonstrated the natural horizontal transfer of these alkaloids through soil as another important contamination route (Nowak et al., 2016; Nowak et al., 2017; Selmar et al., 2019; Chmit et al., 2021; Letsyo et al., 2021; Jiao et al., 2022; Jiao et al., 2023a). On the other hand, in some cases, the occurrence of these toxins could also be due to intentional adulteration by using undisclosed herbs to gain economic advantage. In fact, oregano is one of the most adulterated herbs (Maquet et al., 2021). Due to the high frequency of contamination of these products with TAs and PAs, different authors have developed several analytical procedures for the determination of these alkaloids in aromatic herbs and spices (Kaltner et al., 2020a; Izcara et al., 2020; Kaczyński and Łozowicka, 2020; González-Gómez et al., 2022b; Izcara et al., 2022; González-Gómez et al., 2023a; Peloso et al., 2023). For instance, in the case of TAs, extraction has been performed with acidic water solution (1.1.% HCl) followed by solid-phase extraction (SPE) (González-Gómez et al., 2022b; González-Gómez et al., 2023a). Different novel sorbents based on mesostructured silicas modified with sulfonic groups have been developed for this purpose. The results obtained in these works revealed coriander, basil, fennel and cloves as the products with the highest concentrations of TAs (González-Gómez et al., 2022b; González-Gómez et al., 2023a). For PAs, acidic solvent extraction with 0.05 M H_2SO_4 followed by SPE with cation-exchange sorbents (e.g., SCX and MCX) has been used as sample treatment (Kaltner et al., 2020a; Peloso et al., 2023). However, besides SPE, other techniques such as QuEChERS have successfully been applied to the analysis of PAs in herbs and spices (Izcara et al., 2020; Kaczyński and Łozowicka, 2020; Izcara et al., 2022). In this context, it is worth highlighting those works which have miniaturized the QuEChERS procedure up to 10 times and applied it to the analysis of oregano and other aromatic herbs using PSA or mesostructured silicas as clean-up sorbents (Izcara et al., 2020; Izcara et al., 2022). This miniaturization has allowed improving the QuEChERS strategy, leading to a more sustainable and green method. Nonetheless, despite the technique employed, PA levels exceeding the maximum limit established by regulation for aromatic herbs have been detected in these works, namely, in oregano, marjoram, cumin, thyme, parsley, savory, dill and chive (Kaltner et al., 2020a; Izcara et al., 2020; Kaczyński and Łozowicka, 2020; Izcara et al., 2022; Peloso et al., 2023).

2.2 Determination in cereals and bakery products

Within the period reviewed, food alerts have only been reported for the presence of TAs in this type of products. Like herbs and spices, the contamination is mainly due to cross-contamination during cultivation and harvesting or by natural horizontal transfer. However, these contamination routes are also possible in the case of PAs. Therefore, although no PA alerts have been reported in these products, some works have also addressed the determination of PAs in cereal samples, even in some cases simultaneously together with the analysis of TAs (Dzuman et al., 2020; Bessaire et al., 2021; Letsyo et al., 2021; García-Juan et al., 2023; Rollo et al., 2023). Thus, among the different strategies developed for the analysis of TAs and PAs in these food matrices separate or together, the most common sample preparation procedure has been acidic aqueous solvent extraction followed in some cases by cation-exchange SPE or the QuEChERS method (Basle et al., 2020; Dzuman et al., 2020; González-Gómez et al., 2020; Bessaire et al., 2021; Letsyo et al., 2021; Rausch et al., 2021; Vuković et al., 2022; García-Juan et al., 2023; Rollo et al., 2023). Regarding TAs, atropine and scopolamine ranged between 2.6 and 58.80 μg/kg in different cereal-based products (e.g., teff, buckwheat, sorghum, rice, semolina, corn grits and polenta) (Basle et al., 2020; González-Gómez et al., 2020; Rausch et al., 2021; Vuković et al., 2022). In all cases, contamination by atropine was greater than that by scopolamine. Likewise, scopoline, a low molecular weight TA, was detected in a quinoa sample at 38 μg/kg (Bessaire et al., 2021). However, data of low molecular weight TAs in foods is currently very scarce. On the other hand, trace amounts of PAs were found in maize grown on fields previously dominated with a PA-producing plant (*Chromolaena odorata*) (Letsyo et al., 2021) and 3 PAs were detected in a multigrain baby food sample (García-Juan et al., 2023). Thus, these results suggest the need to also explore the occurrence of PAs in cereal-based products.

2.3 Determination in teas and herbal infusions

These products have also caused many of the food alerts reported for both types of alkaloids. Like herbs and spices, teas and herbal teas are susceptible to contamination due to co-harvesting and natural horizontal transfer. For the determination of these compounds, different strategies have been addressed. On the one hand, some authors have determined the presence of these alkaloids in the dry product (Kwon et al., 2021; Han et al., 2022a; Han et al., 2022b; Jansons et al., 2022; León et al., 2022; Luo et al., 2022; Jiao et al., 2023b; Gumus, 2023; Mateus et al., 2023; Peloso et al., 2023). For PAs, many authors have performed and acidic solvent extraction with H_2SO_4 (0.05–0.1 M) followed by SPE purification with MCX cartridges or dispersive clean-up with GCB, PSA and C18 sorbents (Kwon et al., 2021; Han et al., 2022a; Han et al., 2022b; Jiao et al., 2023b; Peloso et al., 2023). Likewise, QuEChERS has been used for both PAs and TAs (Han et al., 2022b; Jansons et al., 2022; León et al., 2022; Jiao et al., 2023b; Mateus et al., 2023). Regarding miniaturization, alkaline organic extraction followed by solid-phase microextraction (SPME) using molecularly imprinted fibers with cation-exchange interaction was employed for selective recognition of 10 PAs from scented teas (Luo et al., 2022). On the other hand, other works have directly performed the analysis in the prepared infusion samples (Casado et al., 2022b; Han et al., 2022b; González-Gómez et al., 2022c; Martinello et al., 2022; Rizzo et al., 2023a; González-Gómez et al., 2023b; Fernández-Pintor et al., 2023). Analyzing the infusion seems more suitable to avoid overestimation of the real intake and exposure of consumers to these toxins through these beverages, since transfer rate of these alkaloids during brewing is not always complete (Casado et al., 2023). Accordingly, some recent works have assessed the transfer rate of these alkaloids in addition to estimating their content in the product. In these cases, after preparing the infusions, the aqueous extracts have been purified before chromatographic analysis by using different techniques, such as SPE, salting-out assisted liquid-liquid extraction (SALLE) and μSPEed (Casado et al., 2022b; Han et al., 2022b; González-Gómez et al., 2022c; Martinello et al., 2022; Rizzo et al., 2023a; González-Gómez et al., 2023b; Fernández-Pintor et al., 2023). It is worth highlighting this last technique, which it is an improved high-resolution miniaturized SPE that provides more efficiency and faster procedures at a lower overall cost. This miniaturized approach has proved its green potential for both the analysis of PAs and TAs in infusion samples by minimal use of sample, sorbents and solvents (Casado et al., 2022b; González-Gómez et al., 2022c; González-Gómez et al., 2023b; Fernández-Pintor et al., 2023).

2.4 Determination in fruits and vegetables

In 2021, an alert related to the contamination of deep-frozen spinach puree with high concentrations (>1,000 μg/kg) of atropine and scopolamine was notified (Rapid Alert System for Food and Feed, 2023). The occurrence of TAs in spinach products can be due to the similarity with *Datura innoxia* (TA-producing plant) leaves. Likewise, this may also happen in the case of PAs, since the leaves of arugula or rocket are very similar to the leaves of *Senecio vulgaris* (PA-producing plant). Thus, contamination with toxins due to co-harvesting or natural horizontal transfer is highly possible in vegetables, especially in salad mix. On the other hand, all the alerts related to PAs in vegetables have been reported in 2023, specifically in lovage leaf, chives and borage (Rapid Alert System for Food and Feed, 2023). Nonetheless, it is worth noting that borage is a PA-producing plant, which it is intentionally consumed despite its content in PAs. Regarding the literature, the analysis of these alkaloids in vegetable samples is scarce. For instance, TAs have been determined in spinach-based products using QuEChERS (Castilla-Fernández et al., 2021). Atropine and scopolamine were found in 16 out of 66 samples, with concentrations between 0.02 and 8.19 μg/kg, but only one sample exceed the maximum limit established by the EU. More recently, the miniaturized version of QuEChERS has been applied to the determination of TAs in leafy vegetable samples, achieving a greener procedure with reduced amount of samples, solvents and salts without losing extraction efficiency (González-Gómez et al., 2022d). Mixed leafy vegetables were contaminated in a controlled manner with TA-producing plants simulating a real cross-contamination to assess the efficiency of the method and it was then applied to 18 commercial samples. Atropine was detected in 12 samples, whereas scopolamine was detected only in one, with concentrations of 2.7–3.4 μg/kg. In the case of PAs, they have been determined in borage leaves using solvent extraction with 0.05 M H_2SO_4 followed by SPE with MCX cartridges (Peloso et al., 2023). As expected, all borage samples presented PAs (mean concentration 530.8 μg/kg), and only in one the content (3410 μg/kg) exceeded the maximum limit (750 μg/kg).

2.5 Determination in honey and pollen

In the last 3 years, food alerts related to these products have only been notified for high levels of PAs found in pollen (Rapid Alert System for Food and Feed, 2023). Nonetheless, within this period, many authors have addressed the determination of TAs and PAs in honey and pollen samples (Gottschalk et al., 2020; He et al., 2020; Moreira et al., 2020; Romera-Torres et al., 2020; Thompson et al., 2020; Bandini and Spisso, 2021; Valese et al., 2021; Schlappack et al., 2022a; Schlappack et al., 2022b; Guo et al., 2022; Jansons et al., 2022; Kowalczyk and Kwiatek, 2022; Kwon et al., 2022; Martinello et al., 2022; Rizzo et al., 2022; Rizzo et al., 2023a; Peloso et al., 2023; Roncada et al., 2023; Casado et al., 2024b). The results achieved in these works have revealed these products are frequently contaminated with these toxins, detecting levels of PAs and TAs in wide range (0.012–3313 μg/kg). In PA- and TA-producing plants, these alkaloids can be present in flower nectar. Consequently, contamination of pollen and honey with these toxins mainly happens during bee forage. Pollen is normally more contaminated than honey since it is closely related to the plant materials. In contrast, the occurrence of PAs and TAs in honey is due to the accidental or deliberate introduction of pollen in this matrix. For the extraction of these toxins from this type of matrices, most authors have first dissolved the samples in 0.05 M H_2SO_4, since it helps to release the analytes from the matrix and decrease the sample viscosity. Afterwards, for sample purification, different conventional sample preparation procedures have been employed, being SPE the most widely used, followed by QuEChERS (Gottschalk et al., 2020; He et al., 2020; Moreira et al., 2020; Romera-Torres et al., 2020; Schlappack et al., 2022a; Schlappack et al., 2022b; Guo et al., 2022; Jansons et al., 2022; Kowalczyk and Kwiatek, 2022; Kwon et al., 2022; Martinello et al., 2022; Peloso et al., 2023; Roncada et al., 2023). For SPE, cartridges with cation-exchange sorbents (e.g., MCX, SCX) have been preferred. Accordingly, some authors have developed and applied different sulfonated halloysite nanotubes as novel cation-exchange SPE sorbents for this purpose (Schlappack et al., 2022a; Schlappack et al., 2022b). On the other hand, liquid-liquid extraction (LLE) and SALLE have also been explored for the extraction of TAs and PAs, respectively (Thompson et al., 2020; Rizzo et al., 2022; Rizzo et al., 2023a). Regarding miniaturization, a recent work proposed the green simultaneous microextraction of TAs and PAs from honey by µSPEed® in just 3 min (Casado et al., 2024b). Nonetheless, some authors have avoided sample purification by directly dissolving the sample in water o aqueous ammonium hydroxide and injecting it into the chromatographic system (Bandini and Spisso, 2021; Valese et al., 2021). However, this leads to higher matrix interferences that can damage the ionization source in mass spectrometry detection. Likewise, this does not allow preconcentration, what may hinder achieving the sensitivity set by legislation for the analysis of these compounds.

2.6 Determination in food supplements

There have not been many food alerts in this category within the last 3 years, only 2 in 2023 regarding the occurrence of PAs in *Tribulus terrestris* and *Ginkgo biloba* extracts (Rapid Alert System for Food and Feed, 2023). In this period, some authors have proposed strategies for the analysis of PAs in plant-based and honey-derived food supplements (Kaltner et al., 2020b; Picron et al., 2020; Chen et al., 2021; Rizzo et al., 2023a; Rizzo et al., 2023b). In these works, a first solvent extraction with acidic aqueous solution with 0.05 M H_2SO_4 has been performed followed by a purification step by SPE with cation-exchange sorbents or SALLE prior to chromatographic analysis. Nonetheless, Chen et al. (2021) carried a different approach by performing first an aqueous solvent extraction of the samples with 0.1% formic acid followed by basification of the extract and LLE with dichloromethane. The organic phase was then recovered, evaporated and re-dissolved in methanol:water (1:1, v/v) for direct analysis in real-time coupled to MS (DART-MS) (Chen et al., 2021). Nonetheless, a smaller number of PAs were assessed in the samples compared to the previous works with chromatographic separation.

2.7 Determination in snacks

In 2022, only 3 alerts were reported for TAs in this type of products, specifically in corn tortilla chips (Rapid Alert System for Food and Feed, 2023). However, no works analyzing this type of snack have been found in the literature. Nonetheless, TAs have recently been determined in corn puffs (popular extruded snacks made of cornmeal) by QuEChERS (Stojanović et al., 2021). Only atropine was quantified in 22% of the samples analyzed, but although scopolamine was not quantified, it was also detected in 4 samples. It is worth mentioning that authors highlighted the importance of knowing whether TAs were lost during the extrusion process. The effect of food processing on these toxins is also a relevant issue scarcely studied (Casado et al., 2023). Accordingly, another research has evaluated the thermal degradation of TAs in gluten-free corn breadsticks under different conditions (Vera-Baquero et al., 2022). The results obtained indicated degradation of TAs can be up to 65% during the baking process depending on the conditions used. Likewise, atropine and scopolamine have been detected in popcorn samples in a range of 5.3–28.0 and 2.1–6.3 µg/kg, respectively (Vuković et al., 2022). In the case of PAs, they have been analyzed in honey-based candies and snacks (honey breakfast cereals, honey/cereal bars and gingerbreads) (Picron et al., 2020). The extraction was performed with 0.05 M H_2SO_4 followed by SPE with SCX cartridges. Only one-third of the snacks were contaminated, with a maximum level of 0.36 µg/kg, and all the cereal bars were free of PAs. Regarding candies, 54% of the samples showed PA contamination. Nonetheless, the authors mainly attributed the occurrence of PAs in the snacks and candies to the presence of honey as ingredient.

3 Analytical methods for simultaneous determination of TAs and PAs

Table 1 summarizes all the analytical methods developed in the last 3 years that addressed the simultaneous determination of TAs and PAs. These works have mainly been developed for food matrices with high probability of being contaminated with both toxins because of their similar contamination pathways (i.e., herbs and spices, teas and herbal infusions, cereals, honey and pollen). Among the sample preparation techniques, QuEChERS has been the most widely used, since is a very effective procedure when it is necessary to simultaneously extract many analytes of different nature. Nonetheless, other techniques such as SPE, dispersive SPE, solid-liquid extraction or µSPEed have also been applied (Table 1). Regarding the analytical technique, all works have used LC or UHPLC coupled to MS. Accordingly, different analyzers have been used, being the most common the triple quadrupole and those based on the combination of quadrupole and linear ion trap (e.g., Q-Orbitrap or Q-TRAP). Likewise, the ionization source has been in all cases ESI in positive ion mode. For the chromatographic separation different types of C18 columns have been used at a temperature range between 30–50°C. In general, the analysis time in most works ranged between 15 and 20 min. However, in those works using LC instead of UHPLC the analysis time is generally higher, exceeding 1 h in some cases (Table 1). In contrast, it is worth highlighting the work of Dzuman et al. (2020), which achieved the separation of 54 compounds in 22 min (Dzuman et al., 2020).

TABLE 1 Analytical methods published for the simultaneous determination of tropane and pyrrolizidine alkaloids in food samples (2020–2023).

Number of alkaloids	Food matrix	Sample preparation	Analysis	LOD/LOQ	Recoveries (%)	Ref.
26 TAs, 56 PAs	Pea, soy, wheat flour and quinoa	**QuEChERS**: 1 g of the sample in 10 mL H_2O was hand-shaken for a few seconds. Then 10 mL of ACN was added and shaken at 1,500 rpm x 3 min. At that moment, QuEChERS extraction packet (6 g $MgSO_4$, 1.5 g CH_3COONa) was added and was briefly hand-shaken and then shaker at 1,500 rpm x 3 min. After centrifugation (4000g x 10 min), 1 mL of the supernatant was transferred into a 15 mL polypropylene tube and evaporated to dryness under a stream of N_2 at 40°C. Residues were reconstituted with 100 µL of MeOH, followed by addition of 900 µL of water. The mixture was sonicated (10 min). The solution was centrifuged (17000g x 10 min, 4 °C). Finally, the clean extract was injected in the UHPLC-HRMS system	UHPLC Q-Exactive HF-X HRMS Mobile Phase: (A) NH_4HCO_3 in water; (B) NH_4HCO_3 in MeOH. Analysis time: 25 min Column: BEH C18 (150 × 2.1 mm, 1.7 µm) at 50°C	1 µg/kg	31–132% PAs 58–138% TAs	Bessaire et al. (2021)
28 PAs, 2 TAs	Cereal-based food	**QuEChеRS**: 2.5 g of the sample, 30 mL ACN/H_2O (75:25 v/v) containing 0.1% of FA, and QuEChERS extraction packet (1 g $C_6H_8Na_2O_8$, 0.5 g $C_6H_5Na_3O_7.2H_2O$, 1 g NaCl and 4 g of MgSO4) were vigorously shaked by hand (1min) and centrifuged (5000 rpm x 5 min). The supernatant was transferred into a Falcon tube containing (900 mg $MgSO_4$, 150 mg PSA and 150 mg C18). Shaked by hand (1 min) and centrifuged (5000 rpm x 5 min). The supernatant was evaporated reconstituted with 250 µL of MeOH, and then filtered for subsequent LC-MS/MS analysis	HPLC-MS/MS ESI positive ion mode and MRM mode Column: Luna 3u C18 (100 × 2.0 mm, 3 µm) Mobile Phase: (A) 0.1% FA in water; (B) MeOH with 0.1% FA. Analysis time: 32 min	0.02–0.29/0.5–1 µg/kg PAs 0.05–0.04/0.5 µg/kg TA	72–119% PAs 84–117% TAs	García-Juan et al. (2023)
21 PAs, 2 TAs	Cereals and spices	**QuEChеRS**: 1 g of the sample in 10 mL MeOH/H_2O/FA (60:40:0.4 v/v/v) was shaken (30 min) and centrifuged (5000 rpm x 8 min). 1 mL of the extract was pipetted in the QuEChERS kit (150 mg $MgSO_4$ and 25 mg PSA), then the solution was shaken (1 min) and frozen (1 h). After thawing, the extract was centrifuged (5000 rpm x 5 min) and then filtered for subsequent LC-MS/MS analysis	UHPLC-Q-Orbitrap-MS H-ESI positive ion mode and HRMS mode Column: ACQUITY UPLC BEH™ C18 column (2.1x150 mm, 1.7 µm) at 40°C Mobile Phase: (A) 2 mM AF and 0.2% FA in water; (B) 2 mM AF and 0.2% FA in MeOH Analysis time: 22 min	0.2–0.3/0.4–0.8 µg/kg PAs 0.1/0.4 µg/kg TA	73–111% PAs 85–90% TAs	Rollo et al. (2023)

(Continued on following page)

TABLE 1 (*Continued*) Analytical methods published for the simultaneous determination of tropane and pyrrolizidine alkaloids in food samples (2020–2023).

Number of alkaloids	Food matrix	Sample preparation	Analysis	LOD/LOQ	Recoveries (%)	Ref.
21 TAs, 33 PAs	Sorghum, oregano and herbal tea	**SLE:** 1 g of sample with 10 mL of MeOH/H_2O/FA (60:39.6:0.4; v/v) extracted for 30 min. Centrifuged (13.081g x 5 min) and filtered. 2 mL of extract was purified by dSPE (100 mg of sorbent), manual shaked (1 min) and centrifugated (13081g x 5 min). The extract was filtered prior to analysis	UHPLC-QTRAP-MS/MS ESI positive ion mode and MRM mode Column: Luna Omega C18; (150 × 2.1 mm; 1.6 μm) at 50°C Mobile Phase: (A) 2 mM AF + 0.2% FA in water; (B) 2 mM AF + 0.2% FA in MeOH. Analysis time: 22 min	-/1.0–10 μg/kg TA -/0.5–10 μg/kg PAs	78–115% TAs 83–115% PAs	Dzuman et al. (2020)
28 PAs, 2 TAs	Teas and herbs infusions	**QuEChERS**: 1 g of the sample, 30 mL ACN/H_2O (75:25 v/v) containing 0.5% of formic acid, and QuEChERS extraction packet (6 g $MgSO_4$, 1.5 g CH_3COONa) were vigorously stirred (1min) and centrifuged (4500 rpm x 15 min). 10 mL of supernatant were transferred into a QuEChERS d-SPE (1,200 mg $MgSO_4$, 400 mg PSA, 400 mg C18, and 400 mg GCB). Vortexed (1 min) and centrifuged (4500 rpm x 15 min). The supernatant was evaporated reconstituted with 300 µL of a mixture of H_2O/MeOH (95:5 v/v) containing 0.1% of FA, stirred (1 min), and then filtered for subsequent LC-HRMS analysis	HPLC-Q-Orbitrap-MS/MS H-ESI positive mode and PRM mode Column: Hypersil GOLD C18 column (100×2.1 mm, 1.9 µm) at 40°C Mobile Phase: (A) 0.1% of FA in water; (B) 0.1% of FA in MeOH. Analysis time: 15 min	-/5 µg/kg	87–111% PAs 90–111% TAs	León et al. (2022)
25 PAs, 2 TAs	Herbal Teas and Culinary Herbs	**SLE:** 50 mg of solid sample was sonicated in 2.5 mL of MeOH for 30 min and then centrifuged (3000 rpmx15 min). The supernatant was transferred to a 10 mL metered flask. The SLE procedure was repeated 3 more times, the supernatants were combined and adjust to 10 mL with MeOH. Then, 2 mL of the mixture were filtered for subsequent LC-Q-ToF/MS.	HPLC-Q-ToF/MS ESI positive ion mode and MRM mode Column: Poroshell 120 EC-C18 (2.1 x 150 mm, 2.7 µm) at 40°C Mobile Phase: (A) 0.1% FA in water; (B) 0.1% FA in ACN. Analysis time: 65 min	0.105–0.867/ 0.357–2.890 µg/kg PAs 0.160–0.181/ 0.540–0.599 µg/kg TA	81–106% PAs 79–100% TAs	Gumus (2023)
20 PAs, 2 TAs	Honey bee-collected pollen, teas and herbal infusions	*Pollen* **SLE**: 1 g pollen, 10 mL H_2SO_4 0.1 M and 0.5 g zinc dust was extracted for 90 min. The suspension was centrifuged (3700 gx10 min), the supernatant transferred in a tube with ACN (10 mL) and **QuEChERS** extraction salts (0.5 g $C_6H_8Na_2O_8$, 1 g $C_6H_5Na_3O_7.2H_2O$, 1 g NaCl and 4 g $MgSO_4$), the mixture was agitated (3 min) and centrifuged (3700 g × 10 min). 7 mL of supernatant put into a tube with purification salts (0.9 g $MgSO_4$ and 0.15 g PSA). The mixture was shaken (1 min), centrifuged (3 700 gx10 min) and 5 mL of supernatant dried and reconstituted in 1 mL of H_2O (0.1% FA)/ACN (87:13 v/v) and filtered before analysis	UHPLC-MS/MS ESI positive ion mode and MRM mode	Pollen 2.4–5.3/4.0–9.0 µg/kg PAs	Pollen 76–106% PAs	Martinello et al. (2022)

(Continued on following page)

TABLE 1 (*Continued*) Analytical methods published for the simultaneous determination of tropane and pyrrolizidine alkaloids in food samples (2020–2023).

Number of alkaloids	Food matrix	Sample preparation	Analysis	LOD/LOQ	Recoveries (%)	Ref.
		Tea infusions **LLE**: 50 mL of sample was extracted with 270 μL of H_2SO_4 (95–97%) and 1.5 g zinc dust about 90 min. The mixture was centrifuged (3700gx10 min) and poured into C18-SPE (500 mg) and the analytes were eluted with 5 mL 2.5% ammonia in MeOH (x2). The eluate was dried and reconstituted in 11 mL of H_2O (0.1% FA)/ ACN (87:13 v/v) and filtered before analysis	Column: Accucore TM RP-MS (100 × 2.1 mm, 2.6 μm) at 40°C Mobile phase: (A) 0.1% FA in water; (B) MeOH/ ACN (1:1; v/v) Analysis time: 15.7 min	0.1–0.3/0.2–0.5 μg/kg TA Tea infusions: 0.04–0.08/ 0.07–0.14 μg/kg PAs 0.003–0.004/ 0.005–0.007 μg/kg TA	78–91% TAs Tea infusions: 88–116% PAs 94–114% TAs	
15 PAs, 2 TAs	Honey	**SLE**: 10 g of honey with 20 mL of 0.05 M sulphuric acid and 1.5 g zinc dust were shaked (30 min) and centrifuged (4000 g x 10 min). The extract was purified by MCX-SPE (500 mg). eluting with 10 mL EtAc/MeOH/ACN/NH_3/ TEA (8:1:1:0.3:0.1, v/v). The eluate was dried, reconstituted with 0.2 mL H_2O Milli Q and 0.2 mL of MeOH and filtered prior analysis	HPLC-TQ-MS/MS ESI positive ion mode and MRM mode Column: Kinetex C18 (2.6 μm, 100 mm × 4.6 mm) at 30°C Mobile phase: (A) 0.3% FA in water; (B) MeOH/ ACN (2:1, v/v) Analysis time: 26 min	0.05–0.17/ 0.17–0.58 μg/kg PAs 0.11–0.15/ 0.36–0.49 μg/kg TA	81–106% PAs 84–103% TAs	Kowalczyk and Kwiatek (2022)
21 PAs, 2 TAs	Honey	**SLE**: 0.5 g of honey with 2.5 mL of 0.05 M sulphuric acid. **μSPEed**: 4 mg PS/DVB sorbent, elution with 100 μL MeOH and subsequent LC-MS/MS analysis	UHPLC-IT-MS/MS ESI positive ion mode and MRM mode Column: Luna Omega Polar C18 (100 mm × 2.1 mm, 1.6 μm) at 30°C Mobile phase: (A) water containing 0.2% formic acid; (B) MeOH containing 0.2% ammonia Analysis time: 15 min	0.12–0.30/0.40–1.00 μg/kg	72–100% PAs 81–97% TAs	Casado et al. (2024b)

AF, ammonium formiate; C18: octadecyl bonded silica; DAD, diode array detection; dSPE, dispersive solid phase extraction; EC, encapped; ESI, electrospray ionization; FA, formic acid; H-ESI, heated-ESI; HPLC, high performance liquid chromatography; HRMS, high resolution mass spectrometry; IT, ion trap; LLE, Liquid-Liquid extraction; LOD, limit of detection; LOQ, limit of quantification; MCX, mixed-cation-exchange; MeOH, methanol; MRM, multiple reaction monitoring; MS, mass spectrometry; MS/MS, tandem mass spectrometry; PAs, pyrrolizidine alkaloids; PCX, polymeric cation exchange; PRM, product reaction monitoring; PS/DVB, polystyrene-divinylbenzene; Q, single quadrupole; QTRAP, hybrid triple quadrupole-linear ion trap; QuEChERS, quick, easy, cheap, effective, rugged and safe; SCX, strong cation exchange; SLE, Solid-Liquid extraction; SPE, Solid-phase extraction; TAs, Tropane alkaloids; TQ, triple quadrupole; UHPLC, Ultra-high performance liquid chromatography.

4 Conclusions, challenges and expected future trends

Currently, the occurrence of natural toxins is a food safety topic of great relevance. In recent years, the number of alerts notified for TAs and PAs has increased. However, this may be a consequence of greater food control. To date, the strategies carried out for the determination of these alkaloids are mainly based on acid solvent extraction followed by SPE purification with cation-exchange sorbents, or the QuEChERS procedure. However, future works should make efforts to develop green procedures by employing miniaturized alternatives of these techniques to improve sustainability and optimize resources, including the simultaneous analysis of these two types of alkaloids. Likewise, future works should focus on the effect of food processing on these alkaloids, since it is a field scarcely explored. Thus, the evaluation of degradation/transformation products constitutes a research line with interesting future challenges.

Author contributions

NC: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Visualization, Writing–original draft, Writing–review and editing. JG: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Visualization, Writing–original draft, Writing–review and editing. SM-Z: Supervision, Visualization, Writing–review and editing. IS: Funding acquisition, Project administration, Resources, Supervision, Writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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