Journal of Food Composition and Analysis 125 (2024) 105758

Contents lists available at ScienceDirect

Journal of Food Composition and Analysis

journal homepage: www.elsevier.com/locate/jfca

Review article

Comprehensive overview of the analytical methods for determining pyrrolizidine alkaloids and their derived oxides in foods

Adrián Fuente-Ballesteros [a], Ondrej Brabenec [b], Aristeidis S. Tsagkaris [b], Ana M. Ares [a], Jana Hajslova [b], José Bernal [a,*]

[a] Analytical Chemistry Group (TESEA), I.U. CINQUIMA, Faculty of Sciences, University of Valladolid, 47011 Valladolid, Spain
[b] Department of Food Analysis and Nutrition, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Technická 5, 166 28 Prague 6–Dejvice, Prague, Czech Republic

ARTICLE INFO

Keywords:
Chromatography
Food control
Food safety
Herbal infusions
Honey
Pyrrolizidine alkaloids
QuEChERS
Sample preparation
Solid-phase extraction
Solvent extraction

ABSTRACT

Pyrrolizidine alkaloids and their derived oxides are toxins naturally produced by plants when they are exposed to stress factors. In particular, the unsaturated pyrrolizidine alkaloids exhibiting a double bond between the 1,2-positions have caused great interest in recent years since they have pneumotoxic, genotoxic and carcinogenic effects upon ingestion. In this review, focus on their chemical structure justifying their toxicity is provided as well as insight on the recently adopted EU regulatory framework. In addition, to reveal current trends and highlight the research effort in the field, an all-time bibliometric analysis was performed for the first time. It was found that tea, infusions, honey, spices, and cereals are the most common foodstuffs in which pyrrolizidine alkaloids have been detected. In terms of pre-analytical steps, the application of efficient sample preparation is necessary to detect pyrrolizidine alkaloids in complex food matrices. Solvent extraction followed by a clean-up stage, e.g., solid-phase extraction was the most applied option. Focusing on the analytical methods, liquid chromatography combined with various mass spectrometry detectors has been the golden standard in the field. Gas chromatography methods were also applied, but the need for analyte derivatisation has hindered their application. All in all, this review provides an overview on the analysis of pyrrolizidine alkaloids and their derived oxides, comprehensively discussing the up-to-date advances and highlighting the analytical challenges to be faced.

1. Introduction

Foods contain numerous nutrients such as proteins, fats, carbohydrates, vitamins, and minerals that are essential for human growth, development, reproduction, and proper functioning of the immune system. However, they can also contain natural toxic chemical compounds in varying concentrations posing a health risk (Chen et al., 2019; Di Bella et al., 2019; Dusemund et al., 2018; Kristanc & Kreft, 2016; Letsyo et al., 2017; Rivera-Pérez et al., 2021; Xu et al., 2019). Within this context, various food alerts have been reported (Casado et al., 2022)

Abbreviations: **APCI**, Atmospheric Pressure Chemical Ionization; **BfR**, Bundesinstitut für Risikobewertung; **CI**, Chemical Ionization; **C_{18}**, Octadecylsilane; **DLLME**, Dispersive Liquid-liquid Microextraction; **EFSA**, European Food Safety Authority; **EI**, Electron Impact Ionization; **ESI**, Electrospray Ionization; **EU**, European Union; **FID**, Flame-Ionization Detector; **FSANZ**, Food Standards Australia New Zealand; **GC**, Gas Chromatography; **GCB**, Graphitized Carbon Black; **GC-MS**, Gas Chromatography-Mass Spectrometry; **HFBA**, Heptafluorobutyric anhydride; **HILIC**, Hydrophilic Interaction Chromatography; **HPLC**, High Performance Liquid Chromatography; **HPLC-MS**, High Performance Liquid Chromatography-Mass Spectrometry; **HRMS**, High Resolution Mass Spectrometry; **IC-LC**, Ion Exchange Liquid Chromatography; **ISO**, International Organization for Standardization; **IT**, Ion Trap; **LC**, Liquid Chromatography; **LOD**, Limit Of Detection; **MLs**, Maximum Levels; **MOE**, Margin of Exposure; **MRM**, Multiple Reaction Monitoring; **MS/MS**, Tandem Mass Spectrometry; **MSTFA**, N-methyl-N-(trimethylsilyl)trifluoroacetamide; **NMR**, Nuclear Magnetic Resonance; **NPD**, Nitrogen-Phosphorus Detector; **NP-LC**, Normal Phase Liquid Chromatography; **PAs**, Pyrrolizidine Alkaloids; **PANOs**, Pyrrolizidine Alkaloid N-oxides; **PFP**, Pentafluorophenylpropil; **Q**, Single Quadrupole; **QHQ**, Quadrupole-Hexapole-Quadrupole; **QqQ**, Triple Quadrupole; **QToF**, Quadrupole Time-of-Flight; **QuEChERS**, Quick, Easy, Cheap, Effective, Rugged & Safe; **RASFF**, Rapid Alert System for Food and Feed; **RP-LC**, Reverse Phase Liquid Chromatography; **SALLE**, Salting-out Assisted Liquid-Liquid Extraction; **SCX**, Strong-Cation Exchange; **SE**, Solvent Extraction; **SIM**, Single Ion Monitoring; **SPE**, Solid-Phase Extraction; **TLC**, Thin-Layer Chromatography; **UAE**, Ultrasound-Assisted Extraction; **UHPLC**, Ultra High-Performance Liquid Chromatography; **UV**, Ultraviolet; **UV-Vis**, Ultraviolet-Visible; **2D-HPLC**, Two-Dimensional High Performance Liquid Chromatography.

* Corresponding author.
E-mail address: jose.bernal@uva.es (J. Bernal).

https://doi.org/10.1016/j.jfca.2023.105758
Received 14 August 2023; Received in revised form 5 October 2023; Accepted 9 October 2023
Available online 12 October 2023

related to high concentrations of pyrrolizidine alkaloids (PAs) and their derived pyrrolizidine alkaloid N-oxides (PANOs) in different food matrices (Mulder et al., 2018). PAs are secondary metabolites produced by plants and their synthesis is triggered by elevated levels of stress phenomena affecting their development, such as damage caused by herbivorous animals, insects, or microorganisms (Guldiken et al., 2018; Hartmann, 1999; Theis & Lerdau, 2003; Sixto et al., 2019). These natural toxins act as a defence barrier and are produced by a wide variety of plants, commonly belonging to the *Asteraceae*, *Fabaceae*, *Boraginaceae*, *Orchidaceae* and *Apocynaceae* families (Merz & Schrenk, 2016; Prinsloo et al., 2018; Roeder & Wiedenfeld, 2011). PAs/PANOs commonly accumulate in seeds and flowering parts of plants, with lower concentrations in leaves, stems, and roots. The content of PAs in plant matrices depends on a multiple range of factors including species, plant organ, harvest, and storage. These compounds exhibit toxicity when a contaminated food is digested indicating a serious and largely ignored food safety issue. It may have been ignored in the past, but in the last two decades interest has tremendously grown, as is also evident when checking the number of related articles included in this review. Pneumotoxic, genotoxic and carcinogenic responses, liver damage, pulmonary hypertension, cardiac hypertrophy, kidney damage or death may occur, consequences that have been extensively studied (Chen et al., 2010; Chen et al., 2019; Fu et al., 2002; Huxtable, 1990; Wiedenfeld, 2011; Wiedenfeld & Edgar, 2011). For this reason, it is essential to control the presence of PAs in food products by developing sensitive and powerful analytical methods to detect and quantify these compounds at trace levels.

PAs are generally found in plant-derived products such as honey, pollen, tea, herbal teas, food supplements, spices, and aromatic herbs. However, PAs have also been detected in animal products such as milk, meat, and eggs, however, in lower concentration (Casado et al., 2022; Kempf et al., 2010; Lucchetti et al., 2016; Mulder et al., 2015, 2018). The main source of food supply chain contamination is the accidental harvesting of plants containing PAs, but there have also been cases of adulteration, migration through the soil or transfer of metabolites to animal products as a result of feeding (Bodi et al., 2014; Edgar & Smith, 1999; Kaltner et al., 2020; Nowak et al., 2016; Schulz et al., 2015; Selmar et al., 2019a; Selmar al., 2019b). Most of the studies found in the scientific literature focuses on a single food matrix, particularly honey (Edgar et al., 2002; Kempf et al., 2010; Lucchetti et al., 2016). Nevertheless, there are only a few papers that provide a broader view on food matrices and analytical methods for PA determination. In this study, we deliver a holistic review identifying the most contaminated matrices by PAs alongside an expert opinion on the analytical challenges related to PA determination. All this comprehensive information is intended to highlight the importance of analysing these alkaloids since their presence in food has been widely neglected posing a potential threat to human health.

2. Chemical structure and toxicity

To justify the need to monitor the presence of PAs/PANOs in food matrices it is important to understand their toxic potential, which is related to their chemical structure. PAs are based on two partly or fully hydrogenated pyrrole rings fused with a nitrogen heteroatom at position 4 (see Fig. 1); it is a bicyclic base consisting of 2 structural components: a) the necic acid and b) a necine base, which contains a pyrrolizidine ring system. All necine bases have a hydroxymethyl substituent at position 1 and a hydroxyl group at position 7 (Rowell-Rahier et al., 1991; Xia et al., 2008). These compounds can appear in the unoxidized (free form) or in the oxidized form (N-oxides or PANOs; EFSA, 2011; Griffin et al., 2017; Valese et al., 2016). PAs can be divided in two classes according to the existence of a double bond between positions 1 and 2 of the necine base: 1,2-unsaturated PAs and saturated PAs. Furthermore, according to the structures of the necine base, PAs are divided into four groups: retronecine, heliotridine, and otonecine (unsaturated bases) and platynecine (saturated base; Gottschalk et al., 2015; Svečnjak et al., 2021; These et al., 2013; Wang et al., 2005; Zhou et al., 2010; see Fig. 2). Given all the possible chemical combinations of these structures, there could be hundreds of different molecules of natural pyrrolizidine alkaloids. This diversity means that an analyst must accept the challenge of extracting, separating, identifying, and quantifying a wide variety of PAs/PANOs in very different matrices such as plants, teas, infusions, seeds, honey, or pollen, amongst others.

PAs are defined as protoxins (Merz & Schrenk, 2016), because they are inactive molecules that need to be activated to exert their function. In this sense, the correlation between chemical structure and toxicity is justified since the presence of a double bond in the necine base (1, 2-unsaturated PAs) increases the toxicological potency of these compounds (Chen et al., 2019; Lee, 2003) comparing to the weak or non-toxic effect of saturated necine base (Fu et al., 2004; Ruan et al., 2014). The toxicity of PAs derives from prior absorption and subsequent metabolic transformation. Such transformation can occur in multiple ways, the most important being i) hydrolysis, ii) oxidation to give rise to PANOs, and iii) oxidation that generates reactive pyrroles. Such oxidation is performed by the cytochrome P450 enzyme family (EFSA, 2011;

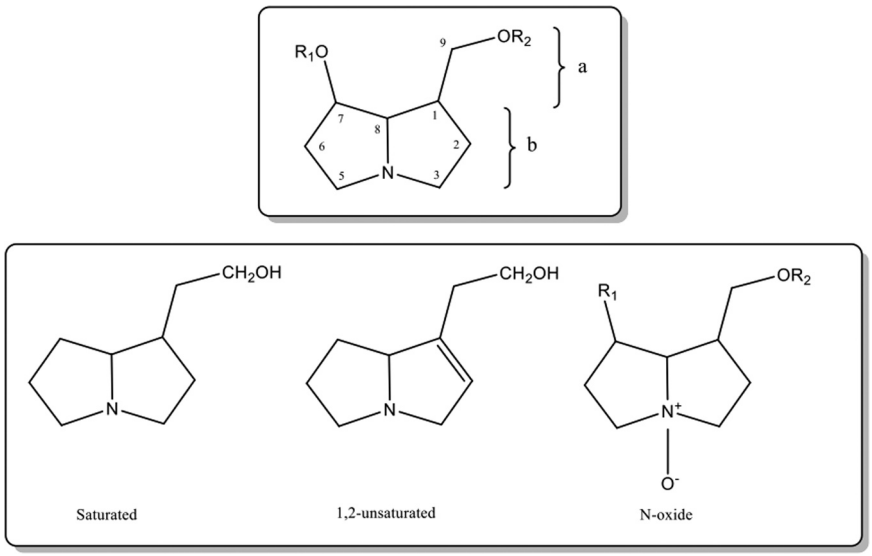

Fig. 1. - Common chemical structure of PAs and its different forms (R_1 and R_2 correspond to different necic acids).

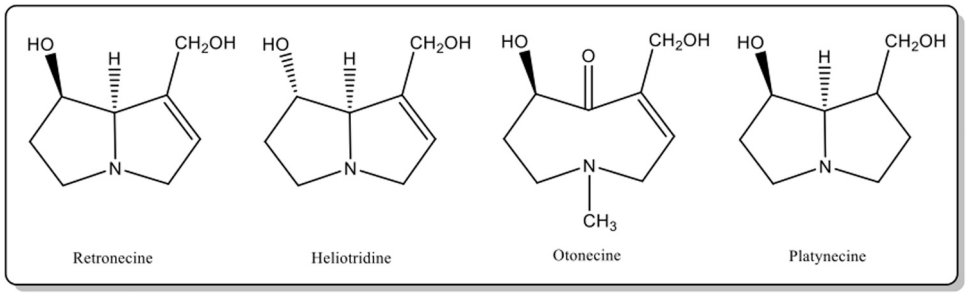

Fig. 2. - Types of PAs according to the necine base.

Merz & Schrenk, 2016; Moreira et al., 2018; Ober & Kaltenegger, 2009). PAs toxicity is influenced by different factors that are primarily based on i) metabolic pathways, ii) characteristics of the individual, and iii) chemical structure of the alkaloid. Toxic PAs are ester alkaloids and can occur as monoesters, open chain diesters and macrocyclic diesters (Wiedenfeld, 2011). For example, PAs that contain a moiety with a cyclic diester are potentially more toxic as they are more potent to form DNA crosslinks (Fu et al., 2004; Martinello et al., 2014; Prakash et al., 1999; Xia et al., 2008). This toxicity reported in different studies around the world, has been generally attributed to hepatotoxic, pneumotoxic, genotoxic, carcinogenic, mutagenic, and tumor-inducing effects (Dusemund et al., 2018; Fu et al., 2002; Kakar et al., 2010; Rasenack et al., 2003; Wiedenfeld, 2011).

3. EU Regulatory framework on PAs/PANOs

Because of the health risk posed by the ingestion of foods contaminated with PAs, the European Food Safety Authority (EFSA) has issued different reports (EFSA, 2007, 2011, 2016, 2017) in which a strong concern has been expressed about these compounds especially in honey, teas, infusions, and food supplements. Worthy to notice is that also national agencies, both European and non-European, e.g., the German Federal Institute for Risk Assessment (BfR, 2011, 2013, 2016, 2020) or Food Standards Australia New Zealand (FSANZ Food Standards Australia New Zealand, 2001) have supported research assessing the toxicity and risk posed by the dietary exposure to PAs. Based on these concerns, the European Commission (EU) recently established maximum levels (MLs) for PAs/PANOs in certain food commodities (see Table 1). In this document, a total of 21 PAs/PANOs compounds, but also 14 (co-eluting) isomers, were selected to be monitored given their toxicity (EU, 2020) and frequent occurrence in food products. Important to highlight is the lack of MLs for honey, despite the regular occurrence of such analytes in this matrix. Instead, the regulatory limits were set for pollen and pollen-based food supplements taking into consideration that PAs originate directly from the plant source. Unfortunately, currently, there is not an international body establishing a common framework for the regulation of PAs in food products resulting in different limits, tolerable daily intakes and maximum doses for different food matrices depending on the source. EFSA uses a Margin of Exposure (MOE) approach for genotoxic carcinogens, i.e., the PAs. Applying this MOE of 10,000, 0.0237 μg/kg body weight per day was established as a reference point for chronic consumption; by contrast the BfR has set a total daily intake of 0.1 μg/kg for non-carcinogenic acute effects. Thus, the two limits apply for different end-points. To face this challenge, we strongly believe that food science community has to work together on collecting data that will allow the establishment of firm and reliable conclusions on the presence of PAs/PANOs in the food chain.

Table 1
Maximum level of concentration (μg/kg) of PAs in foodstuff. Data obtained from Casado et al. (2022c) and data published by the EU (2020).

Food product	Maximum level (μg/kg)[a]
Herbal infusions (dried product) - Rooibos, Anise, Lemon balm, Chamomile, Thyme, Peppermint, Lemon verbena and mixtures exclusively composed of these dried herbs	400
Other herbal infusions (dried product) not included above	200
Tea (*Camellia sinensis*) and flavoured tea (*Camellia sinensis*) (dried product)	150
Tea (*Camellia sinensis*), flavoured tea (*Camellia sinensis*) and herbal infusions for infants and young children (dried product)	75
Tea (*Camellia sinensis*), flavoured tea (*Camellia sinensis*) and herbal infusions for infants and young children (liquid)	1.0
Food supplements containing herbal ingredients including extracts except for pollen-based food supplements, pollen, and pollen products	400
Pollen based food supplements, pollen, and pollen products	500
Dried herbs	400
Borage, lovage, marjoram and oregano (dried) and mixtures exclusively composed of these dried herbs	1000
Borage leaves (fresh, frozen) placed in the market for the final consumer	750
Cumin seeds (seed spice)	400

[a] Refer to the maximum total concentration of pyrrolizidine alkaloids (including N-oxides) that can be found in the corresponding food.

4. Methodology to review the literature

To deliver a comprehensive review, it is of utmost importance to perform an all-time scientometric evaluation of the available literature on PAs/PANOs analysis. It is important to highlight that this is the first study implementing such an approach, in contrast to other recent reviews on the topic reporting no information on how the studied literature was selected. To achieve that both Scopus and Web of Science databases were utilised and the following keywords were used: "pyrrolizidine alkaloid" OR "pyrrolizidine alkaloids" AND "food" OR "honey" OR "tea" OR "milk" OR "meat" OR "eggs" OR "supplements" OR "herbal tea" OR "herbs" OR "infusions" AND "analytical method" OR "extraction" OR "detection" OR "screening" OR "food safety" OR "contamination" OR "sample preparation". The acquired results were limited to original papers written in English and published up to 2022. In total 160 publications out of 405 were recorded, and all their abstracts were read to confirm their relevance towards the review topic. Based on these finding, the following interesting facts were identified. Importantly, an ever-increasing trend towards the publication of analytical methods for PAs/PANOs detection was noticed (see Fig. 3). Worthwhile to mention is that till 2004, there was low publishing activity due to the lack of standards, instrumentation or sensitive analytical methods to study PAs/PANOs. In addition, the applied scientometric approach permitted us to identify the most common matrices in which PAs/PANOs have been detected into (see Fig. 4). In detail, these alkaloids were detected in a higher proportion in plant-derived products than in animal products. The majority of analytical methods detected PAs/PANOs in infusions (40%) and honey and other beehive products (29%). In contrast, matrices such as cereals (15%), milk (6%), eggs (4%) or meat (4%), were investigated in fewer publications. As it will be discussed throughout

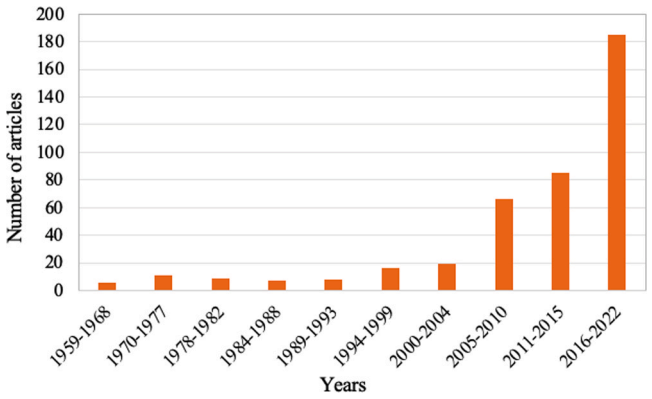

Fig. 3. - Evolution of number of articles for determining pyrrolizidine alkaloids in food products. The methodology employed to review the literature has been explained in detail in Section 4.

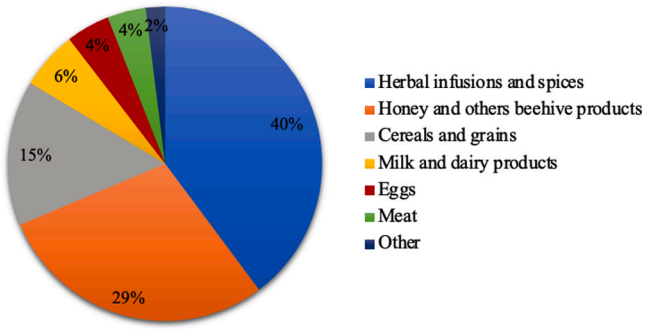

Fig. 4. - Distribution of contaminated food products. The methodology employed to review the literature has been explained in detail in Section 4.

this review, the greatest presence of PAs is found in plant-derived products due to plants are mainly the source of these compounds. In terms of the applied sample preparation methods (see Fig. 5), it was revealed that the most commonly applied sample pre-treatment was solvent extraction (SE; 40%), followed by solid-phase extraction (SPE) (37%) and Quick, Easy, Cheap, Effective, Rugged & Safe extraction (QuEChERS; 17%). Another important characteristic that was feasible to critically evaluate was the preference towards the analytical methods used to measure PAs/PANOs in food matrices (see Fig. 6). Interestingly, it was found that 80% of the studies used chromatographic methods. Chromatographic methods can be hyphenated to a plethora of analytical

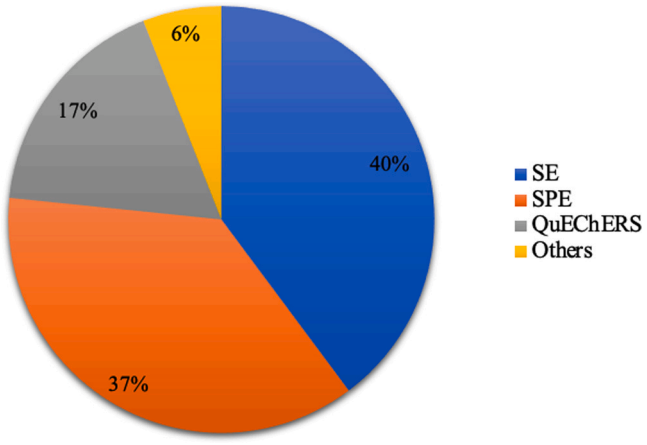

Fig. 5. - Distribution of the sample preparation methods. The methodology employed to review the literature has been explained in detail in Section 4.

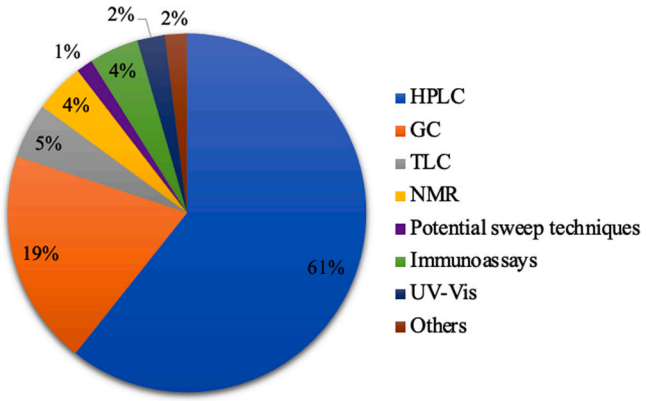

Fig. 6. - Distribution of analytical methods. The methodology employed to review the literature has been explained in detail in Section 4.

detectors and feature high sensitivity and great detectability. High performance liquid chromatography (HPLC; 61%) was by far the most popular analytical method to use followed by gas chromatography (GC; 19%). The advantage of using HPLC is that there is no need for analyte derivatisation (as in the case of GC) as well as the wide spectrum of analytes, in terms of physicochemical characteristics, that can be monitored. Other less common methodologies such as thin-layer chromatography (TLC), nuclear magnetic resonance (NMR), immunoassays, capillary electrophoresis or spectroscopic methods have been also used. In conclusion, a tremendous progress has been reported in terms of analytical methods for PA determination during the last years.

5. Commonly contaminated food matrices

Based on the bibliometric analysis, it was revealed that a plethora of analytical methods have been developed in a wide variety of food matrices such as milk, honey, spices, eggs, dietary supplements, infusions, soy, oils, margarines, leek, meat, cereals, salads, juices and all their derivatives (Adamczak et al., 2013; Avula et al., 2015; Becerra-Jiminez et al., 2013; Kakar et al., 2010; Kempf et al., 2011; Mulder et al., 2018; Yu et al., 2005). Worthy to highlight is the large number of articles found on the presence of these toxins in honey and hive products, spices, teas, aromatic herbs, and food supplements (Kempf et al., 2010; Lucchetti et al., 2016; Mulder et al., 2018; Robertson & Stevens, 2017). On the contrary, it is less frequent to find these contaminants in samples of animal origin, excluding honey, as a transfer phenomenon has to occur from living plants to livestock though the consumed feed (Casado et al., 2022; Mulder et al., 2015; Schrenk et al., 2020; Selmar et al., 2019). Overall, the reported PA concentration greatly varied in the tested food matrices as their occurrence can be affected by the: i) botanical origin, ii) climatic conditions, iii) environmental conditions, iv) plant stage, and v) harvesting method, among many others (Bodi et al., 2014; Kast et al., 2014).

5.1. *Herbal infusions and related matrices*

Undoubtedly, herbal infusions may be related to potential antioxidant and diuretic benefits, upon certain dietary consumption (Chen & Lin, 2015; Dong et al., 2015; Farzaneh & Carvalho, 2015; Hartley et al., 2013). On the downside, this food matrix can be also contaminated or adulterated by PA-producing herbs, so this risk should be carefully monitored and evaluated (Mathon et al., 2014). A characteristic example of that is the comfrey (*Symphytum officinale*) plant, which has been widely used to cure ailments. Nevertheless, comfrey contains significant amounts of PAs showing chronic hepatotoxic effects in humans (Cai et al., 2003; Fu et al., 2002; Yeong et al., 1990). In fact, hundreds of medicinal plants containing PAs have been reported in the literature.

Some of the plant families that frequently appear are Apiaceae, Asteraceae, Boraginaceae, Fabaceae, Lamiaceae, Orchidaceae, and Urticaceae (Asres et al., 2008; Aydin & Letzel, 2013; Bodi et al., 2014; Colegate et al., 2016; Hsieh et al., 2015; Moreira et al., 2018). For example, in a study by Schulz et al. (2015), 169 German commercial samples of medicinal teas were analysed. In another work, the presence of PAs was investigated in a traditional Indonesian beverage brewed from ginger and turmeric. The authors elucidated that there was contamination by alkaloid-producing plants and emphasized that acute toxic effects due to short-term consumption are rather improbable (Suparmi et al., 2020). In addition, there are several studies reporting positive samples originating from various places such as Germany, Ireland, or Switzerland (Bodi et al., 2014; Griffin et al., 2014; Mathon et al., 2014; Schulz et al., 2015). This includes PA contamination in black, green, rooibos, lemon balm, peppermint, chamomile, fennel, nettle, and mixed herbal teas (Bodi et al., 2014; Mulder et al., 2018). Indeed, some studies reveal that rooibos tea is one of the most contaminated matrices mainly due to harvesting (Huybrechts & Callebaut, 2015; Picron et al., 2018). For example, Bodi et al. (2014) reported a mean PAs contamination concentration of 1856.4 μg/kg after analysing 24 rooibos tea samples. The number of contaminated samples indicates that the presence of PAs/-PANOs in this matrix is related to cultivation or harvesting (Schulz et al., 2015). Interestingly, most positive samples found from tea and infusions belong to the senecionine group, a particular type of PA (Bodi et al., 2014; Griffin et al., 2014; Steinhoff, 2019). Finally, it is important to mention liqueurs, elixirs and juices that come from plant and herbal parts and have likewise attracted great attention because of their potential toxic effects on liver cells. This is the case documented by Chmit et al. (2019) who analysed 38 samples of liqueurs, 12 plant elixirs, and 6 different herbal juices. The results showed that 9 liqueurs, 7 elixirs, and 4 juices were positive for PAs.

5.1.1. Aromatic and culinary herbs

PAs/PANOs can also occur in species and culinary herbs, as these products are subject of the same way of contamination already mentioned. As outlined by Casado et al. (2022b) in recent years, the incidents of food alerts reported in the Rapid Alert System for Food and Feed (RASFF) portal regarding the presence of PAs/PANOs in food products were predominantly linked to aromatic herbs. In fact, the highest number of detected cases and alerts issued was from Germany. RASFF (2020) emphasized the significant number of alerts raised due to the relatively high PAs levels found in oregano (ranging from 6660 to 133870 μg/kg). Nevertheless, there is a scarcity of research focused on the detection of PAs/PANOs in spices and aromatic herbs, as opposed to other products like honey, tea, and dietary supplements (Izcara et al., 2020). Similar to spices, aromatic herbs are believed to be susceptible to contamination, not only through co-harvesting with PAs-producing plants or intentional adulteration but also through natural horizontal transfer of PAs/PANOs via the soil (Selmar et al., 2019). In fact, oregano has been identified as one of the most commonly adulterated aromatic herbs (Black et al., 2016). Through adulteration with undisclosed herbs, producers can gain an economic advantage by selling less pure oregano. For instance, Izcara et al. (2020) discovered that all oregano samples tested positive for PAs/PANOs (n = 3); nevertheless, this is a very low number of samples tested. Specifically, europine, europine N-oxide, lasiocarpine, and lasiocarpine N-oxide were detected to varying degrees in all analyzed samples. The average PAs/PANOs concentration was 1254 μg/kg. However, this finding was lower in comparison to prior studies (Kaltner et al., 2020). Kaltner et al. (2020) examined 305 samples from 15 spice and culinary herb matrices (e.g., parsley, oregano, thyme, marjoram, basil, savory, pepper, dill, chive, rosemary, cumin, ginger, curry, caraway, and herbs of provence) originating from 36 countries. Impressively, 58% of the samples contained at least one PA/PANO. The average cumulative content across all samples was 323 μg/kg, with the highest amount of 24.6 mg/kg detected in an oregano sample. Given the substantial number of positive samples reported in the literature, it is imperative to conduct further investigations into the PAs/PANOs content in these food matrices.

5.2. Honey and other beehive products

PAs can be present in flower nectar (Lucchetti et al., 2016; Moreira et al., 2018) indicating the chance of contamination during bee forage, which, ultimately, will result in PA transfer in honey. Therefore, PAs occurrence in honey is primarily related to the existing pollen varieties (Boppré et al., 2005). Pollen present in honey can be introduced accidentally or deliberately (Edgar et al., 2011). Consequently, pollen is the matrix contaminated in higher PA concentrations as it is closely related to the plant materials (Kempf et al., 2010; Kempf et al., 2011; Lucchetti et al., 2016). Similarly, honey is another of the most contaminated products of the beehive, but commonly, the concentration found is insufficient to cause severe health effects, unless the maximum daily intake of honey suggested by regulatory organizations is exceeded (Beales et al., 2004; Betteridge et al., 2005; Kempf et al., 2008; Orantes-Bermejo et al., 2013). The contamination problem is worsening when the rest of the beehive products such as royal jelly, propolis and bee waxes are also contaminated due to the bee proximity to flowers during forage (Kempf et al., 2008). PAs/PANOs have even been found in derivatives such as candies, energy bars, cereals, beverages, or baby food (Cao et al., 2013; Kempf et al., 2011; Mulder et al., 2018; Picron et al., 2020). The presence of these toxins within the hive not only has negative effects on humans as the case reported by Rasenack et al. (2003), but also on the pollinators themselves. PAs affect feeding, communication or may even cause the death of pollinators. Bee larvae are very susceptible and even a low concentration of PAs can be lethal. On the contrary, adult bees are more tolerant to PAs (Lucchetti et al., 2018).

Honey is one of the most important bee products in the field of beekeeping featuring beneficial health-promoting properties, due to its high concentration in bioactive compounds, such as polyphenols (Koulis et al., 2022). However, the presence of PAs/PANOs in its composition poses a risk both for the hive and consumers. There have been several studies reporting the presence of several PAs (senecionine, echimidine and lycopsamine) in honey (Beales et al., 2004; Betteridge et al., 2005; Kempf et al., 2008; Kempf, Wittig, Schönfeld, et al., 2011; Orantes-Bermejo et al., 2013; Zhu et al., 2018). Important factors to consider in honey contamination by PAs/PANOs is the temperature and storage time impacting the metabolite concentration (Kaltner et al., 2018; Kowalczyk et al., 2018). Kowalczyk et al. (2018) performed a stability test of PAs in honey concluding that the extracts can be stored in a refrigerator or freezer for five days without significant changes. In contrast, storage at room temperature resulted in changes in the alkaloid concentrations. Reinhard et al. (2009) claimed that while in plants the PANOs form are generally found, in honey, the free base form of PAs is present. This difference may be due to the reduction reaction taking place in the digestive system of the bees with the presence of weak reducing agents. Considering PA occurrence in honey, positive samples have been found all over the world. A recent study revealed that the 58% of Chinese honeys (n = 255) were contaminated with PAs in the range 0.2–281.1 μg/kg (He et al., 2020). Another investigation identified eight PAs in honey and other hive products from Brazil. Valese et al. (2021) found senecionine and its N-oxide and retrorsine N-oxide in six of the seven samples analysed. Australian honeys were also analysed finding positive samples with PAs contents up to 2.0 μg/g honey (Carpinelli De Jesus et al., 2019).

Regarding pollen, many PAs/PANOs have been monitored with the most frequent analytes being senecionine, echimidine, heliotrine, and lycopsamine (Boppré et al., 2005; Lucchetti et al., 2016; Rollason et al., 2016). However, an important bottleneck of such data is the lack of information on the pollen origin. A solution in that could be the use of melissopalynological analysis, which is able to identify the origin of pollen by identifying its shape and characteristics under a microscope.

Nevertheless, this is a laborious and time-consuming analysis significantly increasing the analysis time (Tsagkaris et al., 2021). In any case, the available information on the occurrence of PAs in pollen is still scarce indicating the need to further investigate this matrix. Last but not the least, it should be noted that the concentration of alkaloids varies greatly and is related to the existing pollen and its botanical origin.

Besides honey, propolis and royal jelly feature also antioxidant, anti-inflammatory, antimicrobial or antiseptic properties (Bankova et al., 2018; Henatsch et al., 2016; Maleki et al., 2019; Park et al., 2019). Their consumption has increased over the years due to their potential health beneficial effects. Unfortunately, despite the nutritional value of these two matrices, studies on propolis and royal jelly are very limited. Based on the current status, pollen and honey are more contaminated than propolis and royal jelly, in which the concentration of PAs/PANOs is significantly lower (Lucchetti et al., 2016; Mulder et al., 2018; Picron et al., 2020). For example, Mulder et al. (2018) found eleven positive pollen samples out of twelve with a mean concentration of 576.0 μg/kg, while the values for propolis and royal jelly were 0.6 and 15.5 μg/kg, respectively. Coelho et al. (2015) detected for the first time echimidine derivatives in aqueous extracts of *Apis mellifera* propolis. Lucchetti et al. (2018) investigated the PA transfer from bee bread to royal jelly using as a control parameter the echimidine concentration in royal jelly produced by bees fed with a doped bee bread. The authors argued that only a small fraction of PAs was transferred to royal jelly by the bee bread.

5.3. Other matrices

5.3.1. Ready-to-eat salads

The case of ready-to-eat salads is a challenging occasion that needs to be emphasized. We should clarify that salads are not being marketed containing PAs-producing plants, but rather that some plants producing PAs have a similar physical appearance to the vegetables in a salad mix. Specifically, arugula or rocket (*Eruca vesicaria*) has attracted attention due to its similarity to the leaves of the senecio plant (*Senecio vulgaris*; Ma et al., 2018; Wiedenfeld, 2011). Picron et al. (2018) conducted a study with seventeen salad samples containing a mix of vegetables, since these are more likely to be contaminated with other species. They found an average PA concentration of 1.13 μg/kg fresh product, mostly attributed to retrorsine, retrorsine N-oxide and seneciphylline N-oxide. Such results are considered relatively high given the high consumption of such pre-packaged food items. Furthermore, none of these samples were properly labelled as arugula, only mixtures of endive, curly endive, chicory, and lamb's lettuce. The authors concluded that, in these cases, the contamination was clearly due to co-harvesting.

5.3.2. Cereals, grains, and their derived products

Some studies have also focused on the analysis of cereals (Chung & Lam, 2018) such as wheat, flour, corn, or soybeans, with several reports of intoxications due to their consumption (Wiedenfeld & Edgar, 2011), e.g., in Ethiopia or Afghanistan (Kakar et al., 2010; Molyneux et al., 2011). For example, Kakar et al. (2010) found toxic doses of PAs in bread samples from Afghanistan causing veno-occlusive disease. The wheat flour used to prepare bread was considered as the most probable contamination source. Besides intoxication cases, several types of cereal samples have been found contaminated. In detail, eleven Korean soybean (*Arachis hypogaea, Cier arietinum, Canavalia gladiate, Phaseolus radiatus* and others) samples were analyzed and traces of intermedine (0.64–1.46 μg/kg) were found (Yoon et al., 2015). Recently, Letsyo et al. (2020) analysed the PAs content in maize (*Zea mays L.*) plant tissues obtaining traces of contaminants in roots, leaves, and kernels. In addition, these authors detected alkaloid levels in soils sampled before planting, which suggests a soil-plant transfer process. Methods have also been developed to detect PAs in wheat (Dzuman et al., 2015) as it is a widely consumed food commodity. Azadbakht & Talavaki, (2003) analysed PAs in wheat and flour samples that could be contaminated by *Senecio* sp. Although the monitored concentrations could not induce acute intoxication, being continuously exposed to PAs in may have cumulative chronic effects, particularly hepatotoxicity. This contamination problem can be further exacerbated when agricultural practices combine grains from different origins (Edgar et al., 2015). This study evidenced that removal of contaminated seeds could still leave PAs residues in uncontaminated grains. In any case, more occurrence data are necessary to be collected in cereals and grains to clarify the potential risk they set to humans.

5.3.3. Milk and dairy products

Another potential source of PA dietary exposure is milk derived from animals that had consumed plants containing these alkaloids. Besides milk, also products derived from it such as yogurt, cheese, or margarines may be contaminated with PAs (Chen et al., 2021; Chung & Lam, 2018; Hoogenboom et al., 2011; Huybrechts & Callebaut, 2015; Yoon et al., 2015). To begin with, the transfer of PAs in cow and sheep milk was already reported by EFSA (EFSA, 2007). Following up, Hoogenboom et al. (2011) and Mulder et al. (2020) investigated the transfer of PAs from contaminated feed to milk. Hoogenboom et al. (2011) found that jacoline can be transferred at a 4% rate while for ragwort, the PA transfer was estimated at 1.4% for jacoline (N-oxide) (Mulder et al., 2020). However, the oxidized forms were not detected in any samples whilst PA occurrence in milk was also reported in the low part-per-billion range (0.05–0.17 μg/L) (Mulder et al., 2018). In another study, 63 samples were analysed and only four were contaminated with PAs (Huybrechts & Callebaut, 2015). These monitored differences may be related to the applied analytic method or the type of the animal feeding. On the contrary, in other recent studies, milk and margarine samples did not contain PAs (reported below LODs; Chen et al., 2021; Yoon et al., 2015). Finally, in-vivo experiments have been performed to investigate the transfer of these contaminants to the milk feeding rat and mice offspring. In fact, it was found that the sucking offspring died with distinct liver lesions after administration of PAs (Edgar et al., 2011).

5.3.4. Meat and eggs

Despite being rather unlikely to find PAs/PANOs residues in meat and egg matrices, still there have been cases reporting their presence. Mulder et al. (2016) investigated the transfer of PAs to food samples by feeding contaminated grasses to hens. A transfer to eggs, in particular to the yolk, and in the meat analysed was observed concluding that contaminated feed ingestion may result in contamination of eggs and meat that could be of concern to consumers. This argument is in line with the study of Chen et al. (2021) who identified that when animals are fed by plants containing PAs, these analytes will be absorbed and transmitted to the food chain, resulting in traces appearing in derived foodstuff. Similar studies have expressed this concern about the transfer of PAs from feed to eggs (Diaz et al., 2014). On the other hand, Mulder et al. (2018) claimed that contamination of milk, eggs, and meat products with significant levels of PAs is unlikely. This may be due to metabolic processes occurring in the animals reducing PA levels. Despite the few scientific papers available in the literature regarding milk, eggs, and meat, it is recommended to maintain relevant research activity aiming to update the occurrence data and clarify PA fate during ingestion (EFSA, 2011).

6. Analytical methods

Different analytical methodologies have been developed aiming to detect and quantify PAs/PANOs in different food matrices (see Supplementary Material, Table 1S). Following the all-time bibliometric analysis presented in Section 4, here special focus is paid in the latest advances related to their analysis. In detail, we will comprehensively discuss the progress on: i) sample preparation strategies including "green chemistry" protocols and ii) analytical methods with strong focus on chromatographic analysis as this is the most widely applied technique. Alternative methods to screen for PAs/PANOs, such as biosensors

(Xiao et al., 2022), will not be further discussed. In any case, these methods have not been extensively used in PAs/PANOs food control due to limitations related to their detectability.

6.1. Sample preparation

Firstly, it is necessary to attain a representative homogenous sample. Sample homogeneity can be achieved through common mixing and grinding procedures. Prior to that, drying using vacuum, oven or freeze-drying can be performed in case of fresh samples while dry plant products are mainly homogenized and milled to a fine powder (Picron et al., 2018).

6.1.1. Solvent extraction

SE is the most common way to extract PAs/PANOs, which is often combined with a purification step employing SPE (Kaltner et al., 2020; Letsyo, 2022; Rivera-Pérez et al., 2021). Considering the hydrophilic PA character and their basic nature, it is common to use polar solvents or acidified aqueous solutions, especially 0.05 M sulfuric acid (Crews et al., 2010; Kwon et al., 2021; Zhang et al., 2008), since both PAs/PANOs species are easily soluble in these solvents. However, other extractants such as hydrochloric acid, formic acid and methanol have also been employed (Bodi et al., 2014; Kaltner et al., 2019; Picron et al., 2018; Shimshoni et al., 2015). Particularly, Shimshoni et al. (2015) observed that acidification of the extractant with aqueous acetic acid improved the recovery values for plant-origin matrices. Worthy to notice is that in older studies, chloroform and dichloromethane were used as the extractant for alkaloids from honey (Deinzer et al., 1977) and eggs (Edgar & Smith, 1999). However, this approach is not used much anymore as these chlorinated solvents do not comply with the principles of "green chemistry" and their side reactions occurring with the PA epoxide groups.

Each food matrix features a diverse composition requiring specific extraction conditions, thus, it is not possible to acquire a universal SE technique. For example, in samples of dried plant material, such as spices, infusions or teas, it may be useful to use ultrasound-assisted extraction (UAE) under acidic conditions (Bodi et al., 2014; Chung & Lam, 2018; Kaczyński & Łozowicka, 2020; Mathon et al., 2014). On other occasions, protocols following International Organization for Standardization (ISO) guidelines have been employed (ISO, 2019) where samples are infused with boiling water or recently salting-out assisted liquid-liquid extraction (SALLE; Rizzo et al., 2022). Following extraction, usually extract purification is performed to ensure proper clean-up of the extract, for example, from other matrix co-extracts such as fats, waxes or terpenes that can adversely affect the analytical procedure.

6.1.2. Solid phase extraction

Due to the complexity of food matrices, after extraction of the PAs/PANOs, an additional clean-up step is usually necessary, with SPE being the most widely used technique (Casado et al., 2022b). However, there were cases that samples were directly analysed without prior purification (Valese et al., 2016). The selection of appropriate SPE cartridges is critical in terms of isolating the PAs/PANOs and achieving acceptable recoveries (Colegate et al. 2005). Among the various available SPE sorbents, reversed-phase (mainly based on octadecylsilane, (C_{18})) and strong-cation exchange (SCX) sorbents have been employed for PAs/-PANOs (Bodi et al., 2014; Chung & Lam, 2018; Griffin et al., 2014; Kaltner et al., 2019; Schulz et al., 2015; Yoon et al., 2015). The use of C_{18} sorbents for SPE clean-up requires an extra neutralization step of the extracts to adjust the pH values in the range 6.0–7.0. This favours analyte interactions with the sorbent stationary phase (Chung & Lam, 2018; Kaltner et al., 2019; Schulz et al., 2015). For example, DSC-C_{18} SPE cartridges were used to clean-up PAs/PANOs extracts from tea and herbal samples. Bodi et al. (2014) conditioned them with 5 ml of methanol and 5 ml of water. The cartridges were then loaded with 10 ml of the sample extract, washed with 6 ml of water and dried under vacuum. Analytes were eluted in two steps with 5 ml each of methanol or 2.5% (1.4 M) ammonia in methanol in case of black or green team samples. SCX cartridges present several advantages because they allow the simultaneous isolation of PAs and PANOs with high yield and less interferences (Cao et al., 2008). Typically, the cartridges are washed with methanol, then conditioned with dilute hydrochloric/sulphuric acid (0.05 M) and the analytes are eluted with methanol and ammonium hydroxide mixtures (Colegate et al., 2005; Bodi et al., 2014). Importantly, this sorbent has been used for honey analysis by diluting the sample with water beforehand to avoid blocking the column (Beales et al., 2004). Using these two types of sorbents, recovery values close to 100% have been obtained (Griffin et al., 2014; Shimshoni et al., 2015) indicating the high selectivity of SPE-based approaches. Other less used types of cartridges include polymeric cation exchange (PCX), graphitized carbon black (GCB), and pentafluorophenyl (PFP; Griffin et al., 2014; Jeong et al., 2021; Ji et al., 2019; Kaltner et al., 2019; Picron et al., 2018). Besides, recent approaches employing sulfonated halloysite nanotubes as cation exchangers for SPE of PAs from honey have also been successfully employed (Schlappack et al., 2022). Overall, SPE generally performs very well in purifying the analytes of interest by selective analyte retention. SPE plays a pivotal role in PAs/PANOs extraction for several reasons. For instance, it facilitates the transformation of sample matrices into a chromatographically compatible form, enhances analyte concentration to boost sensitivity, eliminates interferences to streamline chromatographic procedures increasing quantification accuracy, and safeguards the analytical column against potential contaminants.

6.1.3. QuEChERS

Another popular sample preparation protocol due to its simplicity and ecological (in terms of solvent consumption) characteristics is QuEChERS (Martinello et al., 2017; Qie et al., 2021). Since its introduction, for the simultaneous multi-residue extraction of a wide variety of pesticides from fruit and vegetable samples (Tsagkaris et al. 2019), QuEChERS-based methods have gained widespread popularity, expanding their utility to various sample matrices and analytes. This popularity is attributed to its inherent advantages, including speed, cost-effectiveness, simplicity, user-friendliness, and high-throughput extraction efficiency. However, despite these numerous advantages and its alignment with the principles of green analytical chemistry, QuEChERS method has seen limited use in the determination of PAs compared to other conventional extraction and purification methods like SE or SPE (see Fig. 5). This strategy has predominantly found its use in quantifying PAs in honey samples, with subsequent applications in the analysis of various herbs, particularly oregano, as well as herbal teas (both the dry product and the prepared beverage). Additionally, it has been adapted for the examination of cereals and pseudocereals, including wheat, sorghum, and quinoa. In contrast, its utilization has been somewhat limited in the assessment of legumes (peas and soy) and vegetables (like leek). Furthermore, it has been employed in the analysis of food supplement products such as pollen, as well as in the examination of feed and forage samples (see Suplemental Material, Table 1S).

This strategy involves two steps, firstly, an extraction based on salt dispersion (salting-out effect) to extract and isolate the analytes and, secondly, purifying the extract using different salts and sorbents to remove matrix interferences. Worthwhile to mention is that the solvent type, ratio between sample and extractant, type of salts and quantity, are factors highly vary depending on the matrix highlighting the versatility of QuEChERS-based sample preparation. In addition, efforts have been made to miniaturize this approach reducing sample amount, salt use and organic solvent volume (Casado et al., 2022b). Typically, acidified acetonitrile, methanol, ethyl acetate, and its combinations, have been employed as extraction solvents. Citrate buffers, magnesium sulfate, sodium chloride, trisodium citrate dihydrate, disodium hydrogen citrate sesquihydrate, acetate buffer and sodium acetate have been utilized as

partitioning salts. In terms of clean-up sorbents, GCB, C_{18}, multiwalled carbon nanotubes, graphene, magnetic nanoparticles, zirconia-based sorbents, sol-gel organic-inorganic hybrid sorbents, organic polymers, florisil, alumina, chitosan and ordered mesostructured silicas have been employed (Casado et al., 2022a).

Despite the original QuEChERS method was proposed using acetonitrile, there are many variants of it that showed different performances in terms of recoveries, mainly when acetonitrile extraction procedures are compared to the direct acidified solvent extraction. The most prevalent approach involves the utilization of acidified acetonitrile with various acids, with formic acid being the most widely used, followed by acetic and citric acid. Alternatively, it is common to use mixtures of it with different solvents, including water, methanol, ethyl acetate, dichloromethane, or hexane. In certain studies, it can be replaced by alternative organic solvents like methanol, or combinations of diverse solvents can be used, entirely excluding acetonitrile (Bruzzoniti et al., 2014; Perestrelo et al., 2019).

Several researchers decided to initiate the first step of the QuEChERS procedure by employing acidified acetonitrile, predominantly incorporating various concentrations of formic acid (0.1–2%; Dzuman et al., 2015; Izcara et al., 2022; León et al., 2022; Mol et al., 2011; Qie et al., 2021; Vaclavik et al., 2014). For example, in a study by Kaczyński & Łozowicka (2020), a comparison was made between the extraction of PAs using non-acidified acetonitrile and acetonitrile acidified with 1% formic acid. The findings demonstrated an enhancement in the recovery of the targeted PAs by 6–18% when acetonitrile was acidified. Similarly, Mol et al. (2011) utilized acidified acetonitrile with 1% acetic acid to extract 14 PAs and PANOs from various products, including food supplements, honey, and feed. León et al. (2022) employed a combination of acetonitrile and an acidified aqueous solution containing 0.5% formic acid (75:25, *v/v*) for the extraction of 28 PAs and PANOs from dried teas and herbs. The reason behind the selection of acidified polar solvents or acidified aqueous solutions by authors is the significant polarity of PANOs, which is crucial for simultaneously extracting both PAs and PANOs from the samples (Crews et al., 2010). Vaclavik et al. (2014) also noted that the pH of the matrices played a role in the extraction efficiency of basic analytes such as PAs and PANOs. To face this challenge, they incorporated 2% formic acid into the extraction solvent, resulting in improved recoveries. In another study replacing acetonitrile as the extractant, Dzuman et al. (2020) performed QuEChERS extraction for 33 PAs and PANOs from sorghum, oregano, and mixed herbal tea samples. They utilized a mixture of methanol, water, and formic acid (60:39.6:0.4, *v/v*), which yielded satisfactory analytical results for all the compounds under examination.

6.1.4. Fitness within the "Green Analytical Chemistry" principles

Developing "green" analytical methods focusing on significantly reducing solvent volumes or using green solvents has been booming in recent years (Sajid & Płotka-Wasylka, 2022). It is expected that new environmentally friendly analytical methodologies will be implemented over time, for example, through miniaturization of devices evolving the analysis of PAs/PANOs in food samples in a fast, cheap, and reliable process (Casado et al., 2022a; Izcara et al., 2022). QuEChERS-based extraction has been identified as an "environmentally friendly" (Casado et al., 2022b) protocol achieving a fast and cost-effective sample clean-up (Perestrelo et al., 2019). Similarly, another work has proposed to use the QuEChERS procedure and validated the greenness of the proposed method according to the Analytical Eco-Scale for assessing the greenness of analytical procedures (Gałuszka et al., 2012). This scale penalizes the use of non-green practices such as the use of hazardous reagents, instruments that consume too much energy or waste generation. Another sample preparation technique that could fit under this perspective is dispersive liquid-liquid microextraction (DLLME) because of its low cost, short extraction times and moderate consumption of organic solvents, which reduces its environmental impact (Celano et al., 2019).

6.2. Instrumental techniques

6.2.1. Chromatography

As it was highlighted within the Section 4, HPLC coupled to tandem mass spectrometry (HPLC-MS/MS) and gas chromatography-mass spectrometry (GC-MS) have been the most widely used methods featuring sensitivity, robustness, selectivity and detectability, characteristics necessary within the food control scheme. This is of indispensable importance since MLs were recently established in the EU (see Section 3). Commonly, there is a preference towards HPLC (Dzuman et al., 2015; Kaltner et al., 2019; Mathon et al., 2014) or UHPLC (ultra-high performance liquid chromatography; Bolechová et al., 2015; Chung & Lam, 2018; Cirlini et al., 2019; Huybrechts & Callebaut, 2015; Zhu et al., 2016) because they can measure analytes with various physicochemical characteristics without the need of a derivatization step (as in the case of GC-based analysis). Obviously, this reduces the total analysis time and permits a simpler sample preparation. In terms of ionisation source, electrospray ionization (ESI) in the positive ion mode has been the primary choice for these analytes due to the presence of a nitrogen atom in the chemical structure of PAs/PANOs (Bandini & Spisso, 2021; Chung & Lam, 2017; Cramer et al., 2013; Huybrechts & Callebaut, 2015; Letsyo, 2022). Particularly, ESI is much more suitable than atmospheric pressure chemical ionization (APCI) because of the polar PA/PANO character (Beales et al., 2004). As it is widely known, APCI is commonly used for non-polar or medium-polar compounds with low molecular weight while ESI ionizes more efficiently polar to medium-polar compounds of higher molecular weights. Some authors evaluated both ionization sources, but the ionization of PANOs, which are more polar compounds due to the existence of the oxygen atom, was significantly lower in APCI comparing to ESI (Orantes-Bermejo et al., 2013). Similarly, better results were obtained for these compounds when working in positive ionization mode than in negative mode (Dzuman et al., 2015). Focusing on the utilized chromatographic columns, studies have been performed using different HPLC stationary phases, but most of the works employed C_{18}-type phases (Gray et al., 2004; Griffin et al., 2015; Mroczek et al., 2004; León et al., 2022; see Supplementary Material, Table 1S). On the downside, there are also challenges that need to be faced, specifically co-elution phenomena of analytes can occur as in the case of intermedine/lycopsamine and senecionine/senecivernine as well as their N-oxide derivatives. A striking example of that was Kaltner et al. (2019) who used a C_{18} column, and they did not achieve adequate separation of the analytes neither by gradient optimization nor solvent variations. They observed that the peaks of isomeric groups were not separable and mostly co-eluted. In some cases, this problem can be solved with the use of UHPLC due to high chromatographic power and sensitivity. Adjusting the pH can be another way to face such problems. In detail, the separation of intermedine/lycopsamine and its N-oxides can be achieved with acidic chromatographic conditions, whereas the separation of senecionine/senecivernine and its N-oxides is adequate under basic conditions (Avula et al., 2015). For this reason, works analysing all PAs/PANOs are scarce (Izcara et al., 2020; Kaltner et al., 2019) and frequently chromatographic methods are developed focusing on a limited number of alkaloids.

Hydrophilic interaction chromatography (HILIC) has been used as an alternative (Dzuman et al., 2020) combining characteristics of normal phase liquid chromatography (NP-LC), ion exchange liquid chromatography (IC-LC) and reversed phase liquid chromatography (RP-LC). An approach to face co-elution problems and matrix effects was proposed by van de Schans et al. (2017) and Urban et al. (2019) employing two-dimensional HPLC (2D-HPLC). The use of two dimensions significantly increases the resolving and separation power with respect to the corresponding one-dimensional techniques indicating its applicability in the analysis of structurally similar compounds, such as PA isomers. Van de Schans et al. (2017) developed a 2D-HPLC-MS/MS method that was applied to the analysis of alkaloids in plant extracts using C_{18} columns in

each dimension. Urban et al. (2019) developed and validated a method for a pool of toxins, including PAs such as erucifoline, heliotrine, echimidine, europine, jacobine, retrorsine, senkirkine, amongst others. The analytes were extracted with an acetonitrile: water mixture and injected into 2D-HPLC-MS/MS without any further clean-up. However, the high price of the equipment and software may limit its use. It should be also mentioned that the costs of buying the analytical standards is an issue that also affects all confirmatory methods for PAs.

In the case of GC-based analysis, a derivatization step is necessary to assure that all analytes are volatile. The most typical derivatizing agents are: N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA), phthalic anhydride in pyridine, heptafluorobutyric anhydride (HFBA) or boronate reagents (Kowalczyk & Kwiatek, 2017; Mandić et al., 2015). There are certain challenges related to inherent characteristics of GC-based analysis, specifically, only (semi-)volatile and thermally stable analytes can be detected. In this regard, the GC technique may result in thermal PA decomposition or formation of unnecessary pyrrole-protein adducts. Furthermore, the use of GC makes the analysis of PANOs impossible as these compounds are unstable at the temperatures necessary for volatilization (Ma et al., 2018; Mandić et al., 2015) and a reduction step is necessary. Typically, the columns that have been used in GC analysis range from non-polar (100% dimethyl polysiloxane) to polar stationary phases (14% cyanopropyl-methylpolysiloxane; EFSA, 2011). Another aspect to consider is that when GC analysis is performed, the quantification of PAs is not done individually (analyte by analyte). Instead, the sample is subjected to a reduction process with zinc and lithium aluminium hydride reducing the PANOs to their PA-free form (tertiary base) and quantifying their sum content. Alternatively, since this reduction type is time-consuming, the oxygen-absorbing resin serdoxit can be used requiring only a few minutes (Martinello et al., 2014, 2017, 2022). Other work has also achieved reduction by employing sodium or potassium dithionite (Colegate et al., 2005). As both PAs/-PANOs forms must be considered in the analysis, the reduction is performed to verify determination of both forms. Ultimately, this is a big drawback of GC-based PA/PANO analysis in comparison to liquid chromatography (LC)-based methods.

6.2.2. *Coupling chromatographic methods to various detectors*

A plethora of analytical detectors have been combined to chromatographic separation. Among them, MS/MS is the most suitable due to its high selectivity, specificity, and sensitivity, which allows an unequivocal identification of the analytes by obtaining information on molecular weight, composition, and characteristic fragmentations (Robertson & Stevens, 2017). Multiple reaction monitoring (MRM) has been widely utilized as the detection mode offering high sensitivity. One example was the study by Ji et al. (2019) who used HPLC-MS/MS by performing extraction with PCX-SPE and selecting MRM as the detection mode. The PAs were quantified by external calibration. In contrast, ultraviolet (UV) detection is limited in the analysis of PAs/PANOs because these compounds do not exhibit a characteristic UV spectrum, do not provide structural information (Ma et al., 2018), and lack of a strong chromophore, except from a nonspecific wavelength at 214 nm. Other conventional detectors include the flame ionization detector (FID) or the nitrogen-phosphorus detector (NPD). These detectors were predominantly coupled to GC-systems and can provide semiquantitative results (Oberlies et al., 2004).

Focusing on MS-based detection, single quadrupole (Q) is the most widely used in GC-based method, employing electron impact ionization (EI) as the ionization source and single ion monitoring (SIM) as the detection mode (Ma et al., 2018). The mass spectra formed by necine base signals allow elucidating the molecular structure. Since some complex structures may exhibit low intensity in the mass spectrum, it may be useful to apply chemical ionization (CI) to increase the abundance of ions. Meanwhile, the triple quadrupole (QqQ) analyser has been the most widely used (Cirlini et al., 2019; Griffin et al., 2014; Kaltner et al., 2019; Mathon et al., 2014; Mudge et al., 2015; Picron et al., 2018) due to its high selectivity and sensitivity, especially when LC-based separations are performed. However, other works also described detection with ion trap (IT) analysers and to a lesser extent, Q, and quadrupole-hexapole-quadrupole detectors (QHQ; Bandini & Spisso, 2021; Griffin et al., 2013; Hoogenboom et al., 2011; Vaclavik et al., 2014). A disadvantage of IT is that low-mass fragments are destabilized and not trapped, which is important for PA analysis, because some of the formed fragments follow this trend.

In addition, only a few works employed high-resolution mass spectrometry (HRMS) as detection mode, using Q-Orbitrap (Dzuman et al., 2015; Martinello et al., 2017; Vaclavik et al., 2014) and quadrupole time-of-flight (QToF; Wang et al., 2019) analyzers, which are very suitable for this type of detection providing good results for complex mixtures and high throughput, in terms of analyte number. Compared to traditional QqQ analyzers, the HRMS approach allows retrospective data mining, non-targeted screening, and even post-targeted analysis as well as a reliable quantification and confirmation of theoretically unlimited number of analytes within a single analytical run (Rivera-Pérez et al., 2021; Dzuman et al., 2020). Dzuman et al. (2015) used a Q-Exactive™ high-resolution tandem mass spectrometer equipped with heated electrospray ionization for the identification and quantification of 11 PAs in different food matrices. Furthermore, they pointed out that the availability of multiple confirmatory steps, such as precise mass measurement, isotopic profile analysis, and high-resolution MS/MS fragmentation spectra, greatly enhances the reliability of the acquired data. In general, compiling precise m/z values, high-resolution MS/MS spectra, and retention time data enables the creation of spectral libraries for toxins, such as PAs that could potentially be shared across Q-Orbitrap instruments. In consideration of the lack of commercial analytical standards or in-house synthesized standards, HRMS actually provides more qualitative than quantitative analytical information to the modified toxin analysis (Righetti et al., 2016). From an analytical perspective, PAs/PANOs pose a unique set of challenges. Although there are established protocols for sample preparation and analysis, the detection using HPLC-MS/MS remains particularly demanding. PAs comprise several isomeric substances that cannot be differentiated solely by mass spectrometry because the molecular weight and fragment masses are not distinctive for individual compounds. Even with advanced UHPLC separation technologies, it is often impossible to fully chromatographically separate certain enantiomeric compounds, such as indicine/lycopsamine/echinatine or intermedine-N-oxide/indicine-N-oxide (Dzuman et al., 2020).

7. Conclusions

The PA/PANO analysis in food matrices is an emerging analytical topic of significant importance due to their toxic potential. This was also emphasized by the recently employed regulatory framework in the EU, setting MLs for 21 PAs/PANOs. Based on the all-time bibliometric analysis performed, it was revealed that most of the reviewed methods are focused on the analysis of infusions, herbs, spices, bee products and food supplements. However, it would also be interesting and advisable to determine the presence of these contaminants in other matrices that have not been studied much, such as beverages or bakery products. It is urgent to collect more data on the toxicity and exposure levels of PAs/PANOs as their occurrence information is scarce. Regarding the PA determination, it is essential to develop and validate analytical methods that are sensitive, selective, robust, rapid and that comply as much as possible with the principles of green analytical chemistry. Furthermore, considering the wide variety of PAs/PANOs structures existing in nature, methods must ensure efficient extraction, identification, separation, and quantification of the analytes of interest. It is concluded that HPLC-MS is a better choice than GC-MS, but depending the analysed matrix, a sample clean-up may be required prior to analysis to enhance the analytical performance. This step is usually performed using a SE followed by SPE or QuEChERS-based extraction. Importantly, MS

approaches allow trace level (parts per billion) determination of PAs based on MS/MS identification. Undoubtedly, there are certain challenges that need to be faced in PAs analysis. In detail, identification and quantification has become difficult due to the large number of existing alkaloid structures and the high price of analytical standards, in case they are available. On a positive note, thanks to advances in analytical techniques and greater knowledge of these toxins, more research is being carried out to detect PAs in food matrices and ensure the safety of products for human consumption. In our view, quality systems such as proficiency testing should be further implemented, together with proper vegetation management to minimize the presence of PAs/PANOs in the food chain.

Funding

This work was partially supported by METROFOOD-CZ research infrastructure project (MEYS Grant No: LM2018100) including access to its facilities.

CRediT authorship contribution statement

Adrian Fuente-Ballesteros: Conceptualization, Methodology, Investigation, Writing – original draft, Writing – review & editing. **Ondrej Brabenec:** Conceptualization, Methodology, Investigation, Writing – original draft, Writing – review & editing. **Aristeidis S. Tsagkaris,** Conceptualization, Supervision, Visualization, Writing – original draft, Writing – review & editing. **Ana M. Ares:** Conceptualization, Supervision, Visualization, Writing – original draft, Writing – review & editing. **Jana Hajslova:** Conceptualization, Project administration, Supervision, Visualization, Writing – original draft. Writing – review & editing. **José Bernal:** Conceptualization, Supervision, Visualization, Writing – original draft. Writing – review & editing.

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.

Data Availability

Data will be made available on request.

Acknowledgements

Adrián Fuente-Ballesteros thanks the University of Valladolid for his PhD grant and Erasmus+ KA103for the scholarship received that allows collaboration among international researchers.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jfca.2023.105758.

References

Adamczak, A., Opala, B., Gryszczyńska, A., Buchwald, W., 2013. Content of pyrrolizidine alkaloids in the leaves of coltsfoot (*Tussilago farfara* L.) in Poland. Acta Soc. Bot. Pol. 82 (4), 289–293. https://doi.org/10.5586/asbp.2013.028.

Asres, K., Sporer, F., Wink, M., 2008. Occurrence of pyrrolizidine alkaloids in three Ethiopian *Solanecio* species. Biochem. Syst. Ecol. 36 (5–6), 399–407. https://doi.org/10.1016/j.bse.2007.10.003.

Avula, B., Sagi, S., Wang, Y.H., Zweigenbaum, J., Wang, M., Khan, I.A., 2015. Characterization and screening of pyrrolizidine alkaloids and N-oxides from botanicals and dietary supplements using UHPLC-high resolution mass spectrometry. Food Chem. 178, 136–148. https://doi.org/10.1016/j.foodchem.2015.01.053.

Aydin, A.A., Letzel, T., 2013. Simultaneous investigation of sesquiterpenes, pyrrolizidine alkaloids and N-oxides in Butterbur (*Petasites hybridus*) with an offline 2D-combination of HPLC-UV and LC-MMI-ToF-MS. J. Pharm. Biomed. Anal. 85, 74–82. https://doi.org/10.1016/j.jpba.2013.06.022.

Azadbakht, M., Talavaki, M., 2003. Qualitative and quantitative determination of pyrrolizidine alkaloids of wheat and flour contaminated with *Senecio* in Mazandaran province farms. Iran. J. Pharm. Res. 2 (3), 179–183. https://doi.org/10.22037/IJPR.2010.50.

Bandini, T.B., Spisso, B.F., 2021. Development and validation of an LC-HRMS method for the determination of pyrrolizidine alkaloids and quinolones in honey employing a simple alkaline sample dilution. J. Food Meas. Charact. 15 (5), 4758–4770. https://doi.org/10.1007/s11694-021-01048-9.

Bankova, V., Popova, M., Trusheva, B., 2018. The phytochemistry of the honeybee. Phytochemistry 155, 1–11. https://doi.org/10.1016/j.phytochem.2018.07.007.

Black, C., Haughey, S.A., Chevallier, O.P., Galvin-King, P., Elliott, C.T., 2016. A comprehensive strategy to detect the fraudulent adulteration of herbs: The oregano approach. Food Chem. 210, 551–557. https://doi.org/10.1016/j.foodchem.2016.05.004.

Beales, K.A., Betteridge, K., Colegate, S.M., Edgar, J.A., 2004. Solid-phase extraction and LC-MS analysis of pyrrolizidine alkaloids in honeys. J. Agric. Food Chem. 52 (21), 6664–6672. https://doi.org/10.1021/jf049102p.

Becerra-Jiminez, J., Kuschak, M., Roeder, E., Wiedenfeld, H., 2013. Toxic pyrrolizidinalkaloids as undesired contaminants in food and feed: Degradation of the PAs from *Senecio jacobaea* in silage. Pharmazie 68 (7), 636–639. https://doi.org/10.1691/ph.2013.6518.

Betteridge, K., Cao, Y., Colegate, S.M., 2005. Improved method for extraction and LC-MS analysis of pyrrolizidine alkaloids and their N-oxides in honey: Application to *Echium vulgare* honeys. J. Agric. Food Chem. 53 (6), 1894–1902. https://doi.org/10.1021/jf0480952.

BfR, 2011. Chemical analysis and toxicity of pyrrolizidine alkaloids and assessment of the health risks posed by their occurrence in honey. BfR Opin. 038/2011, 1.

BfR, 2013. Pyrrolizidine alkaloids in herbal teas and teas. BfR Opin. 18 (018), 1–29.

BfR, 2016. Pyrrolizidine alkaloids: Levels in foods should continue to be kept as low as possible. BfR Opin. 030/2016, 1–3.

BfR, 2020. Updated risk assessment on levels of 1,2-unsaturated pyrrolizidine alkaloids (PAs) in foods. BfR Opin. 026, 1–62. https://doi.org/10.17590/20200805-100055.

Bodi, D., Ronczka, S., Gottschalk, C., Behr, N., Skibba, A., Wagner, M., Lahrssen-Wiederholt, M., Preiss-Weigert, A., These, A., 2014. Determination of pyrrolizidine alkaloids in tea, herbal drugs and honey. Food Addit. Contam.: Part A 31, 1886–1895. https://doi.org/10.1080/19440049.2014.964337.

Bolechová, M., Čáslavský, J., Pospíchalová, M., Kosubová, P., 2015. UPLC-MS/MS method for determination of selected pyrrolizidine alkaloids in feed. Food Chem. 170, 265–270. https://doi.org/10.1016/j.foodchem.2014.08.072.

Boppré, M., Colegate, S.M., Edgar, J.A., 2005. Pyrrolizidine alkaloids of Echium vulgare honey found in pure pollen. J. Agric. Food Chem. 53 (3), 594–600. https://doi.org/10.1021/jf0484531.

Bruzzoniti, M.C., Checchini, L., De Carlo, R.M., Orlandini, S., Rivoira, L., Del Bubba, M., 2014. QuEChERS sample preparation for the determination of pesticides and other organic residues in environmental matrices: A critical review. Anal. Bioanal. Chem. 406 (17), 4089–4116. https://doi.org/10.1007/s00216-014-7798-4.

Cai, L., Xing, J., Dong, L., Wu, C., 2003. Application of polyphenylmethylsiloxane coated fiber for solid-phase microextraction combined with microwave-assisted extraction for the determination of organochlorine pesticides in Chinese teas. J. Chromatogr. A 1015 (1–2), 11–21. https://doi.org/10.1016/S0021-9673(03)01328-1.

Cao, Y., Colegate, S.M., Edgar, J.A., 2008. Safety assessment of food and herbal products containing hepatotoxic pyrrolizidine alkaloids: Interlaboratory consistency and the importance of N-oxide determination. Phytochem. Anal. 19 (6), 526–533. https://doi.org/10.1002/pca.1079.

Cao, Y., Colegate, S.M., Edgar, J.A., 2013. Persistence of echimidine, a hepatotoxic pyrrolizidine alkaloid, from honey into mead. J. Food Compos. Anal. 29 (2), 106–109. https://doi.org/10.1016/j.jfca.2012.11.005.

Carpinelli De Jesus, M., Hungerford, N.L., Carter, S.J., Anuj, S.R., Blanchfield, J.T., De Voss, J.J., Fletcher, M.T., 2019. Pyrrolizidine Alkaloids of Blue Heliotrope (*Heliotropium amplexicaule*) and Their Presence in Australian Honey. J. Agric. Food Chem. 67 (28), 7995–8006. https://doi.org/10.1021/acs.jafc.9b02136.

Casado, N., Fernández-Pintor, B., Morante-Zarcero, S., Sierra, I., 2022c. Quick and green microextraction of pyrrolizidine alkaloids from infusions of mallow, calendula, and hibiscus flowers using Ultrahigh-Performance Liquid Chromatography coupled to Tandem Mass Spectrometry analysis. J. Agric. Food Chem. 70 (25), 7826–7841. https://doi.org/10.1021/acs.jafc.2c02186.

Casado, N., Morante-Zarcero, S., Sierra, I., 2022a. Application of the QuEChERS strategy as a useful sample preparation tool for the multiresidue determination of pyrrolizidine alkaloids in food and feed samples: a critical overview. Appl. Sci. 12 (9), 4325. https://doi.org/10.3390/app12094325.

Casado, N., Morante-Zarcero, S., Sierra, I., 2022b. The concerning food safety issue of pyrrolizidine alkaloids: An overview. Trends Food Sci. Technol. 120, 123–139. https://doi.org/10.1016/j.tifs.2022.01.007.

Celano, R., Piccinelli, A.L., Campone, L., Russo, M., Rastrelli, L., 2019. Determination of selected pyrrolizidine alkaloids in honey by dispersive liquid-liquid microextraction and ultrahigh-performance liquid chromatography-tandem mass spectrometry. J. Agric. Food Chem. 67 (31), 8689–8699. https://doi.org/10.1021/acs.jafc.9b02392.

Chen, L., Mulder, P.P.J., Peijnenburg, A., Rietjens, I.M.C.M., 2019. Risk assessment of intake of pyrrolizidine alkaloids from herbal teas and medicines following realistic exposure scenarios. Food Chem. Toxicol. 130, 142–153. https://doi.org/10.1016/j.fct.2019.05.024.

Chen, T., Mei, N., Fu, P.P., 2010. Genotoxicity of pyrrolizidine alkaloids. J. Appl. Toxicol. 30, 183–196. https://doi.org/10.1002/jat.1504.

Chen, Y., Li, L., Xiong, F., Xie, Y., Xiong, A., Wang, Z., Yang, L., 2021. Rapid identification and determination of pyrrolizidine alkaloids in herbal and food samples via direct analysis in real-time mass spectrometry. Food Chem. 334, 127472 https://doi.org/10.1016/j.foodchem.2020.127472.

Chen, Z., Lin, Z., 2015. Tea and human health: biomedical functions of tea active components and current issues. J. Zhejiang Univ. - Sci. B 16 (2), 87–102. https://doi.org/10.1631/jzus.B1500001.

Chmit, M.S., Wahrig, B., Beuerle, T., 2019. Quantitative and qualitative analysis of pyrrolizidine alkaloids in liqueurs, elixirs and herbal juices. Fitoterapia 136, 104172. https://doi.org/10.1016/j.fitote.2019.104172.

Chung, S.W.C., Lam, A.C.H., 2017. Investigation of pyrrolizidine alkaloids including their respective N-oxides in selected food products available in Hong Kong by liquid chromatography electrospray ionisation mass spectrometry. Food Addit. Contam. - Part A Chem., Anal., Control, Expo. Risk Assess. 34 (7), 1184–1192. https://doi.org/10.1080/19440049.2017.1319579.

Chung, S.W.C., Lam, C.H., 2018. Development of an analytical method for analyzing pyrrolizidine alkaloids in different groups of food by UPLC-MS/MS. J. Agric. Food Chem. 66 (11), 3009–3018. https://doi.org/10.1021/acs.jafc.7b06118.

Cirlini, M., Cappucci, V., Galaverna, G., Dall'Asta, C., Bruni, R., 2019. A sensitive UHPLC-ESI-MS/MS method for the determination of tropane alkaloids in herbal teas and extracts. Food Control 105, 285–291. https://doi.org/10.1016/j.foodcont.2019.05.030.

Coelho, G.R., Mendonça, R.Z., De SennaVilar, K., Figueiredo, C.A., Badari, J.C., Taniwaki, N., Namiyama, G., De Oliveira, M.I., Curti, S.P., Evelyn Silva, P., Negri, G., 2015. Antiviral action of hydromethanolic extract of geopropolis from Scaptotrigona postica against antiherpes simplex virus (HSV-1). Evid. -Based Complement. Altern. Med. 296086, 1–10. https://doi.org/10.1155/2015/296086.

Colegate, S.M., Edgar, J.A., Knill, A.M., Lee, S.T., 2005. Solid-phase extraction and HPLC-MS profiling of pyrrolizidine alkaloids and their N-oxides: A case study of *Echium plantagineum*. Phytochem. Anal. 16 (2), 108–119. https://doi.org/10.1002/pca.828.

Colegate, S.M., Gardner, D.R., Betz, J.M., Fischer, O.W., Liede-Schumann, S., Boppré, M., 2016. Pro-toxic 1,2-Dehydropyrrolizidine Alkaloid Esters, Including Unprecedented 10-Membered Macrocyclic Diesters, in the Medicinally-used *Alafia* cf. *caudata* and *Amphineurion marginatum* (Apocynaceae: Apocynoideae: Nerieae and Apocyneae). Phytochem. Anal. 257–276. https://doi.org/10.1002/pca.2624.

Cramer, L., Schiebel, H.M., Ernst, L., Beuerle, T., 2013. Pyrrolizidine alkaloids in the food chain: Development, validation, and application of a new HPLC-ESI-MS/MS sum parameter method. J. Agric. Food Chem. 61 (47), 11382–11391. https://doi.org/10.1021/jf403647u.

Crews, C., Berthiller, F., Krska, R., 2010. Update on analytical methods for toxic pyrrolizidine alkaloids. Anal. Bioanal. Chem. 396, 327–338. https://doi.org/10.1007/s00216-009-3092-2.

Deinzer, M.L., Thomson, P.A., Burgett, D.M., Isaacson, D.L., 1977.). Pyrrolizidine alkaloids: Their occurrence in honey from tansy ragwort (*Senecio jacobaea* L.). Science 195 (4277), 497–499. https://doi.org/10.1126/science.835011.

Diaz, G.J., Almeida, L.X., Gardner, D.R., 2014. Effects of dietary *Crotalaria pallida* seeds on the health and performance of laying hens and evaluation of residues in eggs. Res. Vet. Sci. 97 (2), 297–303. https://doi.org/10.1016/j.rvsc.2014.06.011.

Di Bella, G., Potortì, A.G., Ben Tekaya, A., Beltifa, A., Ben Mansour, H., Sajia, E., Bartolomeo, G., Naccari, C., Dugo, G., Lo Turco, V., 2019. Organic contamination of Italian and Tunisian culinary herbs and spices. J. Environ. Sci. Health - Part B Pestic., Food Contam., Agric. Wastes 54 (5), 345–356. https://doi.org/10.1080/03601234.2019.1571364.

Dong, X., Yang, C., Cao, S., Gan, Y., Sun, H., Gong, Y., Yang, H., Yin, X., Lu, Z., 2015. Tea consumption and the risk of depression: A meta-analysis of observational studies. Aust. N. Z. J. Psychiatry 49, 334–345. https://doi.org/10.1177/0004867414567759.

Dusemund, B., Nowak, N., Sommerfeld, C., Lindtner, O., Lampen, A., 2018. Risk assessment of pyrrolizidine alkaloids in food of plant and animal origin. Food Chem. Toxicol. 115, 63–72. https://doi.org/10.1016/j.fct.2018.03.005.

Dzuman, Z., Jonatova, P., Stranska-Zachariasova, M., Prusova, N., Brabenec, O., Novakova, A., Fenclova, M., Hajslova, J., 2020. Development of a new LC-MS method for accurate and sensitive determination of 33 pyrrolizidine and 21 tropane alkaloids in plant-based food matrices. Anal. Bioanal. Chem. 412 (26), 7155–7167. https://doi.org/10.1007/s00216-020-02848-6.

Dzuman, Z., Zachariasova, M., Veprikova, Z., Godula, M., Hajslova, J., 2015. Multi-analyte high performance liquid chromatography coupled to high resolution tandem mass spectrometry method for control of pesticide residues, mycotoxins, and pyrrolizidine alkaloids. Anal. Chim. Acta 863 (1), 29–40. https://doi.org/10.1016/j.aca.2015.01.021.

Edgar, J.A., Colegate, S.M., Boppré, M., Molyneux, R.J., 2011. Pyrrolizidine alkaloids in food: A spectrum of potential health consequences. Food Addit. Contam. - Part A 28 (3), 308–324. https://doi.org/10.1080/19440049.2010.547520.

Edgar, J.A., Molyneux, R.J., Colegate, S.M., 2015. Pyrrolizidine alkaloids: Potential role in the etiology of cancers, pulmonary hypertension, congenital anomalies, and liver disease. Chem. Res. Toxicol. 28 (1), 4–20. https://doi.org/10.1021/tx500403t.

Edgar, J.A., Roeder, E., Molyneux, R.J., 2002. Honey from plants containing pyrrolizidine alkaloids: A potential threat to health. J. Agric. Food Chem. 50 (10), 2719–2730. https://doi.org/10.1021/jf0114482.

Edgar, J.A., Smith, L.W., 1999. Transfer of pyrrolizidine alkaloids into eggs: Food safety implications. ACS Symp. . Ser. 745, 118–128. https://doi.org/10.1021/bk-2000-0745.ch008.

EFSA, 2007. Opinion of the Panel on contaminants in the food chain [CONTAM] related to pyrrolizidine alkaloids as undesirable substances in animal feed. EFSA J. 447, 1–51.

EFSA, 2011. Scientific Opinion on Pyrrolizidine alkaloids in food and feed. EFSA J. 9 (11), 1–134. https://doi.org/10.2903/j.efsa.

EFSA, 2016. Dietary exposure assessment to pyrrolizidine alkaloids in the European population European. EFSA 14 (8), e04572. https://doi.org/10.2903/j.efsa.2016.4572.

EFSA, 2017. Risks for human health related to the presence of pyrrolizidine alkaloids in honey, tea, herbal infusions and food supplements. EFSA J. 15, 4908. https://doi.org/10.2903/j.efsa.2017.4908.

EU, C.R., 2020. Regulation of maximum levels of pyrrolizidine alkaloids in certain foodstuffs - 2020/2040. Off. J. Eur. Union (Issue 11 diciembre de 2020). https://doi.org/10.2903/j.efsa.2011.2406.

Farzaneh, V., Carvalho, I.S., 2015. A review of the health benefit potentials of herbal plant infusions and their mechanism of actions. Ind. Crops Prod. 65, 247–258. https://doi.org/10.1016/j.indcrop.2014.10.057.

FSANZ (Food Standards Australia New Zealand), 2001. Pyrrolizidine Alkaloids in Food - A Toxicological Review and Risk Assessment. Aust. N. Z. Food Auth. 2, 1–16.

Fu, P.P., Xia, Q., Lin, G., Chou, M.W., 2004. Pyrrolizidine Alkaloids - Genotoxicity, Metabolism Enzymes, Metabolic Activation, and Mechanisms. Drug Metab. Rev. 36 (1), 1–55. https://doi.org/10.1081/DMR-120028426.

Fu, P.P., Yang, Y.-C., Xia, Q., Chou, M.W., Cui, Y.Y., Lin, G., 2002. Pyrrolizidine Alkaloids - Tumorigenic Components in Chinese Herbal Medicines and Dietary Supplements. J. Food Drug Anal. 10, 198–211. https://doi.org/10.38212/2224-6614.2743.

Gałuszka, A., Migaszewski, Z.M., Konieczka, P., Namieśnik, J., 2012. Analytical Eco-Scale for assessing the greenness of analytical procedures. TrAC - Trends Anal. Chem. 37, 61–72. https://doi.org/10.1016/j.trac.2012.03.013.

Gottschalk, C., Ronczka, S., Preiß-weigert, A., Ostertag, J., Klaffke, H., Schafft, H., Lahrssen-wiederholt, M., 2015. Pyrrolizidine alkaloids in natural and experimental grass silages and implications for feed safety. Anim. Feed Sci. Technol. 207, 253–261. https://doi.org/10.1016/j.anifeedsci.2015.06.014.

Gray, D.E., Porter, A., O'Neill, T., Harris, R.K., Rottinghaus, G.E., 2004. A rapid cleanup method for the isolation and concentration of pyrrolizidine alkaloids in comfrey root. J. AOAC Int. 87 (5), 1049–1057. https://doi.org/10.1093/jaoac/87.5.1049.

Griffin, C.T., Danaher, M., Elliott, C.T., Glenn Kennedy, D., Furey, A., 2013. Detection of pyrrolizidine alkaloids in commercial honey using liquid chromatography-ion trap mass spectrometry. Food Chem. 136 (3–4), 1577–1583. https://doi.org/10.1016/j.foodchem.2012.02.112.

Griffin, C.T., Gosetto, F., Danaher, M., Sabatini, S., Furey, A., 2014. Investigation of targeted pyrrolizidine alkaloids in traditional Chinese medicines and selected herbal teas sourced in Ireland using LC-ESI-MS/MS. Food Addit. Contam. - Part A 31 (5), 940–961. https://doi.org/10.1080/19440049.2014.900193.

Griffin, C.T., Mitrovic, S.M., Danaher, M., Furey, A., 2015. Development of a fast isocratic LC-MS/MS method for the high-throughput analysis of pyrrolizidine alkaloids in Australian honey. Food Addit. Contam. - Part A Chem., Anal., Control, Expo. Risk Assess. 32 (2), 214–228. https://doi.org/10.1080/19440049.2014.996789.

Griffin, C.T., Sheehan, A., Danaher, M., Furey, A., 2017. Pyrrolizidine alkaloids in food: analytical, toxicological and health considerations. Anal. Food Toxins Toxic. 1–2 (4), 267–318. https://doi.org/10.1002/9781118992685.ch10.

Guldiken, B., Ozkan, G., Catalkaya, G., Ceylan, F.D., Ekin Yalcinkaya, I., Capanoglu, E., 2018. Phytochemicals of herbs and spices: Health versus toxicological effects. Food Chem. Toxicol. 119, 37–49. https://doi.org/10.1016/j.fct.2018.05.050.

Hartley, L., Flowers, N., Holmes, J., Clarke, A., Stranges, S., Hooper, L., Rees, K., 2013. Green and black tea for the primary prevention of cardiovascular disease. Cochrane Database Syst. Rev. 6. https://doi.org/10.1002/14651858.CD009934.pub2.

Hartmann, T., 1999. Chemical ecology of pyrrolizidine alkaloids. Planta 207, 483–495. https://doi.org/10.1007/s004250050508.

Henatsch, D., Wesseling, F., Kross, K.W., Stokroos, R.J., 2016. Honey and beehive products in otorhinolaryngology: a narrative review. Clin. Otolaryngol. 41 (5), 519–531. https://doi.org/10.1111/coa.12557.

He, Y., Zhu, L., Ma, J., Wong, L., Zhao, Z., Ye, Y., Fu, P.P., Lin, G., 2020. Comprehensive investigation and risk study on pyrrolizidine alkaloid contamination in Chinese retail honey. Environ. Pollut. 267, 115542 https://doi.org/10.1016/j.envpol.2020.115542.

Hoogenboom, L.A.P., Mulder, P.P.J., Zeilmaker, M.J., van den Top, H.J., Remmelink, G. J., Brandon, E.F.A., Klijnstra, M., Meijer, G.A.L., Schothorst, R., van Egmond, H.P., 2011. Carry-over of pyrrolizidine alkaloids from feed to milk in dairy cows. Food Addit. Contam. - Part A 28 (3), 359–372. https://doi.org/10.1080/19440049.2010.547521.

Hsieh, C.H., Chen, H.W., Lee, C.C., He, B.J., Yang, Y.C., 2015. Hepatotoxic pyrrolizidine alkaloids in Emilia sonchifolia from Taiwan. J. Food Compos. Anal. 42, 1–7. https://doi.org/10.1016/j.jfca.2015.01.020.

Huxtable, R.J., 1990. Activation and pulmonary toxicity of pyrrolizidine alkaloids. Pharmacol. Ther. 47 (3), 371–389. https://doi.org/10.1016/0163-7258(90)90063-8.

Huybrechts, B., Callebaut, A., 2015. Pyrrolizidine alkaloids in food and feed on the Belgian market. Food Addit. Contam. - Part A Chem., Anal., Control, Expo. Risk Assess. 32 (11), 1939–1951. https://doi.org/10.1080/19440049.2015.1086821.

ISO. (2019). *ISO 3103:2019; Tea — Preparation of liquor for use in sensory tests* .

Izcara, S., Casado, N., Morante-Zarcero, S., Pérez-Quintanilla, D., Sierra, I., 2022. Miniaturized and modified QuEChERS method with mesostructured silica as clean-up sorbent for pyrrolizidine alkaloids determination in aromatic herbs. Food Chem. 380, 132189 https://doi.org/10.1016/j.foodchem.2022.132189.

Izcara, S., Casado, N., Morante-Zarcero, S., Sierra, I., 2020. A miniaturized QuEChERS method combined with ultrahigh liquid chromatography coupled to tandem mass spectrometry for the analysis of pyrrolizidine alkaloids in oregano samples. Foods 9 (9). https://doi.org/10.3390/foods9091319.

Jeong, S.H., Choi, E.Y., Kim, J., Lee, C., Kang, J., Cho, S., Ko, K.Y., 2021. LC-ESI-MS/MS Simultaneous Analysis Method Coupled with Cation-Exchange Solid-Phase Extraction for Determination of Pyrrolizidine Alkaloids on Five Kinds of Herbal Medicines. J. AOAC Int. 104 (6), 1514–1525. https://doi.org/10.1093/jaoacint/qsab098.

Ji, Y.Bin, Wang, Y.S., Fu, T.T., Ma, S.Q., Qi, Y.D., Si, J.Y., Sun, D.A., Liao, Y.H., 2019. Quantitative analysis of pyrrolizidine alkaloids in Gynura procumbens by liquid chromatography–tandem quadrupole mass spectrometry after enrichment by PCX solid-phase extraction. Int. J. Environ. Anal. Chem. 99 (11), 1090–1102. https://doi.org/10.1080/03067319.2019.1616705.

Kaczyński, P., Łozowicka, B., 2020. A novel approach for fast and simple determination pyrrolizidine alkaloids in herbs by ultrasound-assisted dispersive solid phase extraction method coupled to liquid chromatography-tandem mass spectrometry. J. Pharm. Biomed. Anal. 187, 113351 https://doi.org/10.1016/j.jpba.2020.113351.

Kakar, F., Akbarian, Z., Leslie, T., Mustafa, M.L., Watson, J., Van Egmond, H.P., Omar, M. F., Mofleh, J., 2010. An outbreak of hepatic veno-occlusive disease in Western Afghanistan associated with exposure to wheat flour contaminated with pyrrolizidine alkaloids. J. Toxicol. 2010, 1–7. https://doi.org/10.1155/2010/313280.

Kaltner, F., Kukula, V., Gottschalk, C., 2020. Screening of food supplements for toxic pyrrolizidine alkaloids. J. Fur Verbrauch. Und Lebensm. 15 (3), 237–243. https://doi.org/10.1007/s00003-020-01296-9.

Kaltner, F., Rychlik, M., Gareis, M., Gottschalk, C., 2018. Influence of storage on the stability of toxic pyrrolizidine alkaloids and their N-Oxides in peppermint tea, hay, and honey. J. Agric. Food Chem. 66 (20), 5221–5228. https://doi.org/10.1021/acs.jafc.7b06036.

Kaltner, F., Rychlik, M., Gareis, M., Gottschalk, C., 2020. Occurrence and risk assessment of pyrrolizidine alkaloids in spices and culinary herbs from various geographical origins. Toxins 12 (3), 1–16. https://doi.org/10.3390/toxins12030155.

Kaltner, F., Stiglbauer, B., Rychlik, M., Gareis, M., Gottschalk, C., 2019. Development of a sensitive analytical method for determining 44 pyrrolizidine alkaloids in teas and herbal teas via LC-ESI-MS/MS. Anal. Bioanal. Chem. 411 (27), 7233–7249. https://doi.org/10.1007/s00216-019-02117-1.

Kast, C., Dübecke, A., Kilchenmann, V., Bieri, K., Böhlen, M., Zoller, O., Beckh, G., Lüllmann, C., 2014. Analysis of Swiss honeys for pyrrolizidine alkaloids. J. Apic. Res. 53 (1), 75–83. https://doi.org/10.3896/IBRA.1.53.1.07.

Kempf, M., Beuerle, T., Bühringer, M., Denner, M., Trost, D., Von Der Ohe, K., Bhavanam, V.B.R., Schreier, P., 2008. Pyrrolizidine alkaloids in honey: Risk analysis by gas chromatography-mass spectrometry. Mol. Nutr. Food Res. 52 (10), 1193–1200. https://doi.org/10.1002/mnfr.200800051.

Kempf, M., Heil, S., Haßlauer, I., Schmidt, L., von der Ohe, K., Theuring, C., Reinhard, A., Schreier, P., Beuerle, T., 2010. Pyrrolizidine alkaloids in pollen and pollen products. Mol. Nutr. Food Res. 54 (2), 292–300. https://doi.org/10.1002/mnfr.200900289.

Kempf, M., Wittig, M., Reinhard, A., von der Ohe, K., Blacquière, T., Raezke, K.P., Michele, R., Schreier, P., Beuerle, T., 2011. Pyrrolizidine alkaloids in honey: Comparison of analytical methods. Food Addit. Contam. - Part A 28 (3), 332–347. https://doi.org/10.1080/19440049.2010.521772.

Kempf, M., Wittig, M., Schönfeld, K., Cramer, L., Schreier, P., Beuerle, T., 2011. Pyrrolizidine alkaloids in food: Downstream contamination in the food chain caused by honey and pollen. Food Addit. Contam. - Part A 28 (3), 325–331. https://doi.org/10.1080/19440049.2010.521771.

Koulis, G.A., Tsagkaris, A.S., Katsianou, P.A., Gialouris, P.L.P., Martakos, I., Stergiou, F., Fiore, A., Panagopoulou, E.I., Karabournioti, S., Baessmann, C., van der Borg, N., Dasenaki, M.E., Proestos, C., Thomaidis, N.S., 2022. Thorough Investigation of the Phenolic Profile of Reputable Greek Honey Varieties: Varietal Discrimination and Floral Markers Identification Using Liquid Chromatography–High-Resolution Mass Spectrometry. Molecules 27 (14), 1–17. https://doi.org/10.3390/molecules27144444.

Kowalczyk, E., Kwiatek, K., 2017. Determination of pyrrolizidine alkaloids in selected feed materials with gas chromatography-mass spectrometry. Food Addit. Contam. - Part A 34 (5), 853–863. https://doi.org/10.1080/19440049.2017.1302099.

Kowalczyk, E., Sieradzki, Z., Kwiatek, K., 2018. Determination of pyrrolizidine alkaloids in honey with sensitive gas chromatography-mass spectrometry method. Food Anal. Methods 11 (5), 1345–1355. https://doi.org/10.1007/s12161-017-1115-8.

Kristanc, L., Kreft, S., 2016. European medicinal and edible plants associated with subacute and chronic toxicity part I: Plants with carcinogenic, teratogenic and endocrine-disrupting effects. Food Chem. Toxicol. 92, 150–164. https://doi.org/10.1016/j.fct.2016.04.007.

Kwon, Y., Koo, Y., Jeong, Y., 2021. Determination of pyrrolizidine alkaloids in teas using liquid chromatography–tandem mass spectrometry combined with rapid-easy extraction. Foods 10 (10), 2250. https://doi.org/10.3390/foods10102250.

Lee, W.M., 2003. Drug-Induced Hepatotoxicity. N. Engl. J. Med. 349, 474–485. https://doi.org/10.1056/NEJMra021844.

León, N., Miralles, P., Yusà, V., Coscollà, C., 2022. A green analytical method for the simultaneous determination of 30 tropane and pyrrolizidine alkaloids and their N-oxides in teas and herbs for infusions by LC-Q-Orbitrap HRMS. Journal of Chromatography A 1666, 462835. https://doi.org/10.1016/j.chroma.2022.462835.

Letsyo, E., 2022. High-performance counter-current chromatography purification and off-line mass spectrometry monitoring and identification of pyrrolizidine alkaloid markers of tropical Ghanaian honey. J. Sep. Sci. 45 (4), 960–967. https://doi.org/10.1002/jssc.202100718.

Letsyo, E., Adams, Z.S., Dzikunoo, J., Asante-Donyinah, D., 2020. Uptake and accumulation of pyrrolizidine alkaloids in the tissues of maize (*Zea mays* L.) plants from the soil of a 4-year-old Chromolaena odorata dominated fallow farmland. Chemosphere, 128669. https://doi.org/10.1016/j.chemosphere.2020.128669.

Letsyo, E., Jerz, G., Winterhalter, P., Beuerle, T., 2017. Toxic pyrrolizidine alkaloids in herbal medicines commonly used in Ghana. J. Ethnopharmacol. 202, 154–161. https://doi.org/10.1016/j.jep.2017.03.008.

Lucchetti, M.A., Glauser, G., Kilchenmann, V., Dübecke, A., Beckh, G., Praz, C., Kast, C., 2016. Pyrrolizidine Alkaloids from Echium vulgare in Honey Originate Primarily from Floral Nectar. J. Agric. Food Chem. 64 (25), 5267–5273. https://doi.org/10.1021/acs.jafc.6b02320.

Lucchetti, M.A., Kilchenmann, V., Glauser, G., Praz, C., Kast, C., 2018. Nursing protects honeybee larvae from secondary metabolites of pollen. Proc. R. Soc. B: Biol. Sci. 285 (1875), 20172849. https://doi.org/10.1098/rspb.2017.2849.

Ma, C., Liu, Y., Zhu, L., Ji, H., Song, X., Guo, H., Yi, T., 2018. Determination and regulation of hepatotoxic pyrrolizidine alkaloids in food: A critical review of recent research. Food Chem. Toxicol. 119, 50–60. https://doi.org/10.1016/j.fct.2018.05.037.

Maleki, V., Jafari-Vayghan, H., Saleh-Ghadimi, S., Adibian, M., Kheirouri, S., Alizadeh, M., 2019. Effects of Royal jelly on metabolic variables in diabetes mellitus: A systematic review. Complement. Ther. Med. 43, 20–27. https://doi.org/10.1016/j.ctim.2018.12.022.

Mandić, B.M., Vlajić, M.D., Trifunović, S.S., Simić, M.R., Vujisić, L.V., Vučković, I.M., Novaković, M.M., Nikolić-Mandić, S.D., Tešević, V.V., Vajs, V.V., Milosavljević, S.M., 2015. Optimisation of isolation procedure for pyrrolizidine alkaloids from Rindera umbellata Bunge. Nat. Prod. Res. 29 (9), 887–890. https://doi.org/10.1080/14786419.2014.991929.

Martinello, M., Borin, A., Stella, R., Bovo, D., Biancotto, G., Gallina, A., Mutinelli, F., 2017. 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. 234, 295–302. https://doi.org/10.1016/j.foodchem.2017.04.186.

Martinello, M., Cristofoli, C., Gallina, A., Mutinelli, F., 2014. Easy and rapid method for the quantitative determination of pyrrolizidine alkaloids in honey by ultra performance liquid chromatography-mass spectrometry: An evaluation in commercial honey. Food Control 37 (1), 146–152. https://doi.org/10.1016/j.foodcont.2013.09.037.

Martinello, M., Manzinello, C., Gallina, A., Mutinelli, F., 2022. In-house validation and application of UHPLC-MS/MS method for the quantification of pyrrolizidine and tropane alkaloids in commercial honey bee-collected pollen, teas and herbal infusions purchased on Italian market in 2019–2020 referring to recent Europe. Int. J. Food Sci. Technol. 1–12. https://doi.org/10.1111/ijfs.15567.

Mathon, C., Edder, P., Bieri, S., Christen, P., 2014. Survey of pyrrolizidine alkaloids in teas and herbal teas on the Swiss market using HPLC-MS/MS. Anal. Bioanal. Chem. 406, 7345–7354. https://doi.org/10.1007/s00216-014-8142-8.

Merz, K.H., Schrenk, D., 2016. Interim relative potency factors for the toxicological risk assessment of pyrrolizidine alkaloids in food and herbal medicines. Toxicol. Lett. 263, 44–57. https://doi.org/10.1016/j.toxlet.2016.05.002.

Mol, H.G.J., van Dam, R.C.J., Zomer, P., Mulder, P.P.J., 2011. Screening of plant toxins in food, feed and botanicals using full-scan high-resolution (Orbitrap) mass spectrometry. Food Addit. Contam. - Part A 28 (10), 1405–1423. https://doi.org/10.1080/19440049.2011.603704.

Molyneux, R.J., Gardner, D.L., Colegate, S.M., Edgar, J.A., 2011. Pyrrolizidine alkaloid toxicity in livestock: A paradigm for human poisoning? Food Addit. Contam. - Part A 28 (3), 293–307. https://doi.org/10.1080/19440049.2010.547519.

Moreira, R., Pereira, D.M., Valentão, P., Andrade, P.B., 2018. Pyrrolizidine alkaloids: Chemistry, pharmacology, toxicology and food safety. Int. J. Mol. Sci. 19 (6) https://doi.org/10.3390/ijms19061668.

Mroczek, T., Ndjoko, K., Głowniak, K., Hostettmann, K., 2004. On-line structure characterization of pyrrolizidine alkaloids in *Onosma stellulatum* and *Emilia coccinea* by liquid chromatography-ion-trap mass spectrometry. J. Chromatogr. A 1056, 91–97. https://doi.org/10.1016/j.chroma.2004.08.086.

Mudge, E.M., Jones, A.M.P., Brown, P.N., 2015. Quantification of pyrrolizidine alkaloids in North American plants and honey by LC-MS: single laboratory validation. Food Addit. Contam. - Part A 32 (12), 2068–2074. https://doi.org/10.1080/19440049.2015.1099743.

Mulder, P.P.J., Klijnstra, M.D., Goselink, R.M.A., van Vuuren, A.M., Cone, J.W., Stoopen, G., Hoogenboom, R.L.A.P., 2020. Transfer of pyrrolizidine alkaloids from ragwort, common groundsel and viper's bugloss to milk from dairy cows. Food Addit. Contam. - Part A 37 (11), 1906–1921. https://doi.org/10.1080/19440049.2020.1798028.

Mulder, P.P.J., de Witte, S.L., Stoopen, G.M., van der Meulen, J., van Wikselaar, P.G., Gruys, E., Groot, M.J., Hoogenboom, R.L.A.P., 2016. Transfer of pyrrolizidine alkaloids from various herbs to eggs and meat in laying hens. Food Addit. Contam. - Part A 33 (12), 1826–1839. https://doi.org/10.1080/19440049.2016.1241430.

Mulder, P.P.J., López, P., Castelari, M., Bodi, D., Ronczka, S., Preiss-Weigert, A., These, A., 2018. Occurrence of pyrrolizidine alkaloids in animal- and plant-derived food: results of a survey across Europe. Food Addit. Contam. - Part A 35 (1), 118–133. https://doi.org/10.1080/19440049.2017.1382726.

Mulder, P.P.J., Sánchez, P.L., These, A., Preiss-Weigert, A., Castellari, M., 2015. Occurrence of pyrrolizidine alkaloids in food. EFSA Support. Publ. 12 (8) https://doi.org/10.2903/sp.efsa.2015.en-859.

Nowak, M., Wittke, C., Lederer, I., Klier, B., Kleinwächter, M., Selmar, D., 2016. Interspecific transfer of pyrrolizidine alkaloids: An unconsidered source of contaminations of phytopharmaceuticals and plant derived commodities. Food Chem. 213, 163–168. https://doi.org/10.1016/j.foodchem.2016.06.069.

Ober, D., Kaltenegger, E., 2009. Pyrrolizidine alkaloid biosynthesis, evolution of a pathway in plant secondary metabolism. Phytochemistry 70, 1687–1695. https://doi.org/10.1016/j.phytochem.2009.05.017.

Oberlies, N.H., Kim, N.-C., Brine, D.R., Collins, B.J., Handy, R.W., Sparacino, C.M., Wani, M.C., Wall, M.E., 2004. Analysis of herbal teas made from the leaves of comfrey (*Symphytum officinale*): reduction of N-oxides results in order of magnitude increases in the measurable concentration of pyrrolizidine alkaloids. Public Health Nutr. 7 (7), 919–924. https://doi.org/10.1079/phn2004624.

Orantes-Bermejo, F.J., Serra Bonvehí, J., Gómez-Pajuelo, A., Megías, M., Torres, C., 2013.). Pyrrolizidine alkaloids: their occurrence in Spanish honey collected from purple viper's bugloss (*Echium* spp.). Food Addit. Contam. - Part A 30 (10), 1799–1806. https://doi.org/10.1080/19440049.2013.817686.

Park, M.J., Kim, B.Y., Park, H.G., Deng, Y., Yoon, H.J., Choi, Y.S., Lee, K.S., Jin, B.R., 2019. Major royal jelly protein 2 acts as an antimicrobial agent and antioxidant in royal jelly. J. Asia-Pac. Entomol. 22 (3), 684–689. https://doi.org/10.1016/j.aspen.2019.05.003.

Perestrelo, R., Silva, P., Porto-Figueira, P., Pereira, J.A.M., Silva, C., Medina, S., Câmara, J.S., 2019. QuEChERS - Fundamentals, relevant improvements, applications and future trends. Anal. Chim. Acta 1070, 1–28. https://doi.org/10.1016/j.aca.2019.02.036.

Picron, J.F., Herman, M., Van Hoeck, E., Goscinny, S., 2018. Analytical strategies for the determination of pyrrolizidine alkaloids in plant based food and examination of the transfer rate during the infusion process. Food Chem. 266, 514–523. https://doi.org/10.1016/j.foodchem.2018.06.055.

Picron, J.F., Herman, M., Van Hoeck, E., Goscinny, S., 2020. Monitoring of pyrrolizidine alkaloids in beehive products and derivatives on the Belgian market. Environ. Sci. Pollut. Res. 27 (6), 5693–5708. https://doi.org/10.1007/s11356-019-04499-2.

Prakash, A.S., Pereira, T.N., Reilly, P.E.B., Seawright, A.A., 1999. Pyrrolizidine alkaloids in human diet. Mutat. Res. - Genet. Toxicol. Environ. Mutagen. 443, 53–67. https://doi.org/10.1016/S1383-5742(99)00010-1.

Prinsloo, G., Nogemane, N., Street, R., 2018. The use of plants containing genotoxic carcinogens as foods and medicine. Food Chem. Toxicol. 116, 27–39. https://doi.org/10.1016/j.fct.2018.04.009.

Qie, M., Li, S., Guo, C., Yang, S., Zhao, Y., 2021. Study of the occurrence of toxic alkaloids in forage grass by liquid chromatography tandem mass spectrometry. J. Chromatogr. A 1654, 462463. https://doi.org/10.1016/j.chroma.2021.462463.

Rasenack, R., Müller, C., Kleinschmidt, M., Rasenack, J., Wiedenfeld, H., 2003. Veno-occlusive disease in a fetus caused by pyrrolizidine alkaloids of food origin. Fetal Diagn. Ther. 18, 223–225. https://doi.org/10.1159/000070799.

RASFF. (2020). Food and feed safety alters. Available online at: ⟨https://ec.europa.eu/food/safety/rasff_en⟩ (Accessed 1 october 2023).

Reinhard, A., Janke, M., von der Ohe, W., Kempf, M., Theuring, C., Hartmann, T., Schreier, P., Beuerle, T., 2009. Feeding deterrence and detrimental effects of pyrrolizidine alkaloids fed to honey bees (*Apis mellifera*). J. Chem. Ecol. 35 (9), 1086–1095. https://doi.org/10.1007/s10886-009-9690-9.

Righetti, L., Paglia, G., Galaverna, G., Dall'Asta, C., 2016. Recent advances and future challenges in modified mycotoxin analysis: Why HRMS has become a key instrument in food contaminant research. Toxins 8 (12), 361. https://doi.org/10.3390/toxins8120361.

Rivera-Pérez, A., Romero-González, R., Garrido Frenich, A., 2021. Determination and Occurrence of Alkenylbenzenes, Pyrrolizidine and Tropane Alkaloids in Spices, Herbs, Teas, and Other Plant-derived Food Products Using Chromatographic Methods: Review from 2010–2020. Food Rev. Int. 1–27. https://doi.org/10.1080/87559129.2021.1929300.

Rizzo, S., Celano, R., Campone, L., Rastrelli, L., Piccinelli, A.L., 2022. Salting-out assisted liquid-liquid extraction for the rapid and simple simultaneous analysis of pyrrolizidine alkaloids and related N-oxides in honey and pollen. J. Food Compos. Anal. 108, 104457 https://doi.org/10.1016/j.jfca.2022.104457.

Robertson, J., Stevens, K., 2017. Pyrrolizidine alkaloids: Occurrence, biology, and chemical synthesis. Nat. Prod. Rep. 34, 62–89. https://doi.org/10.1039/c5np00076a.

Roeder, E., Wiedenfeld, H., 2011. Pyrrolizidine alkaloids in plants used in the traditional medicine of Madagascar and the Mascarene islands. Pharmazie 66 (9), 637–647. https://doi.org/10.1691/ph.2011.1572.

Rollason, V., Spahr, L., Escher, M., 2016. Severe liver injury due to a homemade flower pollen preparation in a patient with high CYP3A enzyme activity: A case report. Eur. J. Clin. Pharmacol. 72 (4), 507–508. https://doi.org/10.1007/s00228-015-1986-9.

Rowell-Rahier, M., Witte, L., Ehmke, A., Hartmann, T., Pasteels, J.M., 1991. Sequestration of plant pyrrolizidine alkaloids by chrysomelid beetles and selective transfer into the defensive secretions. Chemoecology 2, 41–48. https://doi.org/10.1007/BF01240665.

Ruan, J., Liao, C., Ye, Y., Lin, G., 2014. Lack of metabolic activation and predominant formation of an excreted metabolite of nontoxic platynecine-type pyrrolizidine alkaloids. Chem. Res. Toxicol. 27 (1), 7–16. https://doi.org/10.1021/tx4004159.

Sajid, M., Płotka-Wasylka, J., 2022. Green analytical chemistry metrics: A review. Talanta 238, 123046. https://doi.org/10.1016/j.talanta.2021.123046.

Schlappack, T., Weidacher, N., Huck, C.W., Bonn, G.K., Rainer, M., 2022. Effective Solid Phase Extraction of Toxic Pyrrolizidine Alkaloids from Honey with Reusable Organosilyl-Sulfonated Halloysite Nanotubes. Separations 9 (10), 270. https://doi.org/10.3390/separations9100270.

Schrenk, D., Gao, L., Lin, G., Mahony, C., Mulder, P.P.J., Peijnenburg, A., Pfuhler, S., Rietjens, I.M.C.M., Rutz, L., Steinhoff, B., These, A., 2020. Pyrrolizidine alkaloids in food and phytomedicine: Occurrence, exposure, toxicity, mechanisms, and risk assessment - A review. Food Chem. Toxicol. 136, 111107 https://doi.org/10.1016/j.fct.2019.111107.

Schulz, M., Meins, J., Diemert, S., Zagermann-Muncke, P., Goebel, R., Schrenk, D., Schubert-Zsilavecz, M., Abdel-Tawab, M., 2015. Detection of pyrrolizidine alkaloids in German licensed herbal medicinal teas. Phytomedicine 22 (6), 648–656. https://doi.org/10.1016/j.phymed.2015.03.020.

Selmar, D., Radwan, A., Hijazin, T., Abouzeid, S., Yahyazadeh, M., Lewerenz, L., Kleinwächter, M., Nowak, M., 2019. Horizontal Natural Product Transfer: Intriguing Insights into a Newly Discovered Phenomenon. J. Agric. Food Chem. 67 (32), 8740–8745. https://doi.org/10.1021/acs.jafc.9b03619.

Selmar, D., Wittke, C., Beck-von Wolffersdorff, I., Klier, B., Lewerenz, L., Kleinwächter, M., Nowak, M., 2019. Transfer of pyrrolizidine alkaloids between living plants: A disregarded source of contaminations. Environ. Pollut. 248, 456–461. https://doi.org/10.1016/j.envpol.2019.02.026.

Shimshoni, J.A., Duebecke, A., Mulder, P.P.J., Cuneah, O., Barel, S., 2015. 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. 32 (12), 2058–2067. https://doi.org/10.1080/19440049.2015.1087651.

Sixto, A., Niell, S., Heinzen, H., 2019. Straightforward determination of pyrrolizidine alkaloids in honey through simplified methanol extraction (QuPPE) and LC-MS/MS modes. ACS Omega 4 (27), 22632–22637. https://doi.org/10.1021/acsomega.9b03538.

Steinhoff, B., 2019. Pyrrolizidine alkaloid contamination in herbal medicinal products: Limits and occurrence. Food Chem. Toxicol. 130, 262–266. https://doi.org/10.1016/j.fct.2019.05.026.

Suparmi, S., Mulder, P.P.J., Rietjens, I.M.C.M., 2020. Detection of pyrrolizidine alkaloids in jamu available on the Indonesian market and accompanying safety assessment for human consumption. Food Chem. Toxicol. 138, 111230 https://doi.org/10.1016/j.fct.2020.111230.

Svečnjak, L., Nunes, F.M., Matas, R.G., Cravedi, J.P., Christodoulidou, A., Rortais, A., Saegerman, C., 2021. Validation of analytical methods for the detection of beeswax adulteration with a focus on paraffin. Food Control 120, 107503. https://doi.org/10.1016/j.foodcont.2020.107503.

Theis, N., Lerdau, M., 2003. The evolution of function in plant secondary metabolites. Int. J. Plant Sci. 164 (3) https://doi.org/10.1086/374190.

These, A., Bodi, D., Ronczka, S., Lahrssen-wiederholt, M., Preiss-weigert, A., 2013. Structural screening by multiple reaction monitoring as a new approach for tandem mass spectrometry: presented for the determination of pyrrolizidine alkaloids in plants. Anal. Bioanal. Chem. 405, 9375–9383. https://doi.org/10.1007/s00216-013-7365-4.

Tsagkaris, A.S., Nelis, J.L.D., Ross, G.M.S., Jafari, S., Guercetti, J., Kopper, K., Zhao, Y., Rafferty, K., Salvador, J.P., Migliorelli, D., Salentijn, G.I., Campbell, K., Marco, M.P., Elliot, C.T., Nielen, M.W.F., Pulkrabova, J., Hajslova, J., 2019. Critical assessment of recent trends related to screening and confirmatory analytical methods for selected food contaminants and allergens. TrAC - Trends Anal. Chem. 121, 115688 https://doi.org/10.1016/j.trac.2019.115688.

Tsagkaris, A.S., Koulis, G.A., Danezis, G.P., Martakos, I., Dasenaki, M., Georgiou, C.A., Thomaidis, N.S., 2021. Honey authenticity: analytical techniques, state of the art and challenges. RSC Adv. 11 (19), 11273–11294. https://doi.org/10.1039/d1ra00069a.

Urban, M., Hann, S., Rost, H., 2019. Simultaneous determination of pesticides, mycotoxins, tropane alkaloids, growth regulators, and pyrrolizidine alkaloids in oats and whole wheat grains after online clean-up via two-dimensional liquid chromatography tandem mass spectrometry. J. Environ. Sci. Health - Part B Pestic., Food Contam., Agric. Wastes 54 (2), 98–111. https://doi.org/10.1080/03601234.2018.1531662.

Vaclavik, L., Krynitsky, A.J., Rader, J.I., 2014. Targeted analysis of multiple pharmaceuticals, plant toxins and other secondary metabolites in herbal dietary supplements by ultra-high performance liquid chromatography-quadrupole-orbital ion trap mass spectrometry. Anal. Chim. Acta 810, 45–60. https://doi.org/10.1016/j.aca.2013.12.006.

Valese, A.C., Daguer, H., Muller, C.M.O., Molognoni, L., da Luz, C.F.P., de Barcellos Falkenberg, D., Gonzaga, L.V., Brugnerotto, P., Gorniak, S.L., Barreto, F., Fett, R., Costa, A.C.O., 2021. Quantification of pyrrolizidine alkaloids in *Senecio brasiliensis*, beehive pollen, and honey by LC-MS/MS. J. Environ. Sci. Health - Part B Pestic., Food Contam., Agric. Wastes 56 (7), 685–694. https://doi.org/10.1080/03601234.2021.1943257.

Valese, A.C., Molognoni, L., de Sá Ploêncio, L.A., de Lima, F.G., Gonzaga, L.V., Górniak, S.L., Daguer, H., Barreto, F., Oliveira Costa, A.C., 2016. A fast and simple LC-ESI-MS/MS method for detecting pyrrolizidine alkaloids in honey with full validation and measurement uncertainty. Food Control 67, 183–191. https://doi.org/10.1016/j.foodcont.2016.02.050.

van de Schans, M.G.M., Blokland, M.H., Zoontjes, P.W., Mulder, P.P.J., Nielen, M.W.F., 2017. Multiple heart-cutting two dimensional liquid chromatography quadrupole time-of-flight mass spectrometry of pyrrolizidine alkaloids. J. Chromatogr. A 1503, 38–48. https://doi.org/10.1016/j.chroma.2017.04.059.

Wang, T., Frandsen, H.L., Christiansson, N.R., Rosendal, S.E., Pedersen, M., Smedsgaard, J., 2019. Pyrrolizidine alkaloids in honey: Quantification with and without standards. Food Control 98, 227–237. https://doi.org/10.1016/j.foodcont.2018.11.033.

Wang, Y., Fu, P.P., Chou, M.W., 2005. Metabolic Activation of the Tumorigenic Pyrrolizidine Alkaloid, Retrorsine, Leading to DNA Adduct Formation *In Vivo*. Int. J. Environ. Res. Public Health 2 (1), 74–79. https://doi.org/10.3390/ijerph2005010074.

Wiedenfeld, H., 2011. Plants containing pyrrolizidine alkaloids: toxicity and problems. Food Addit. Contam. 28, 282–292. https://doi.org/10.1080/19440049.2010.541288.

Wiedenfeld, H., Edgar, J., 2011. Toxicity of pyrrolizidine alkaloids to humans and ruminants. Phytochem. Rev. 10 (1), 137–151. https://doi.org/10.1007/s11101-010-9174-0.

Xiao, Y., Yi, H., Wang, G., Chen, S., Li, X., Wu, Q., Zhang, S., Deng, K., He, Y., Yang, X., 2022. Electrochemiluminescence sensor for point-of-care detection of pyrrolizidine alkaloids. Talanta 249, 123645. https://doi.org/10.1016/j.talanta.2022.123645.

Xia, Q., Yan, J., Chou, M.W., Fu, P.P., 2008. Formation of DHP-derived DNA adducts from metabolic activation of the prototype heliotridine-type pyrrolizidine alkaloid, heliotrine. Toxicol. Lett. 178, 77–82. https://doi.org/10.1016/j.toxlet.2008.02.008.

Xu, J., Wang, W., Yang, X., Xiong, A., Yang, L., Wang, Z., 2019. Pyrrolizidine alkaloids: An update on their metabolism and hepatotoxicity mechanism. Liver Res. 3 (3–4), 176–184. https://doi.org/10.1016/j.livres.2019.11.004.

Yeong, M.L., Swinburn, B., Kennedy, M., Nicholson, G., 1990. Hepatic veno-occlusive disease associated with comfrey ingestion. J. Gastroenterol. Hepatol. 5, 211–214. https://doi.org/10.1111/j.1440-1746.1990.tb01827.x.

Yoon, S.H., Kim, M.S., Kim, S.H., Park, H.M., Pyo, H., Lee, Y.M., Lee, K.T., Hong, J., 2015. Effective application of freezing lipid precipitation and SCX-SPE for determination of pyrrolizidine alkaloids in high lipid foodstuffs by LC-ESI-MS/MS. J. Chromatogr. B 992, 56–66. https://doi.org/10.1016/j.jchromb.2015.04.007.

Yu, L., Xu, Y., Feng, H., Li, S.F.Y., 2005. Separation and determination of toxic pyrrolizidine alkaloids in traditional Chinese herbal medicines by micellar electrokinetic chromatography with organic modifier. Electrophoresis 26, 3397–3404. https://doi.org/10.1002/elps.200500233.

Zhang, F., Wang, C.H., Wang, W., Chen, L.X., Ma, H.Y., Zhang, C.F., Zhang, M., Bligh, S. W.A., Wang, Z.T., 2008. Quantitative analysis by HPLC-MS2 of the Pyrrolizidine alkaloid adonifoline in *Senecio scandens*. Phytochem. Anal. 19 (1), 25–31. https://doi.org/10.1002/pca.1009.

Zhou, Y., Li, N., Choi, F.F., Qiao, C., Song, J., Li, S., Liu, X., Cai, Z., Fu, P.P., Lin, G., Xu, H., 2010. A new approach for simultaneous screening and quantification of toxic pyrrolizidine alkaloids in some potential pyrrolizidine alkaloid-containing plants by using ultra performance liquid chromatography – tandem quadrupole mass spectrometry. Anal. Chim. Acta 681, 33–40. https://doi.org/10.1016/j.aca.2010.09.011.

Zhu, L., Ruan, J.Q., Li, N., Fu, P.P., Ye, Y., Lin, G., 2016. A novel ultra-performance liquid chromatography hyphenated with quadrupole time of flight mass spectrometry method for rapid estimation of total toxic retronecine-type of pyrrolizidine alkaloids in herbs without requiring corresponding standards. Food Chem. 194, 1320–1328. https://doi.org/10.1016/j.foodchem.2014.11.093.

Zhu, L., Wang, Z., Wong, L., He, Y., Zhao, Z., Ye, Y., Fu, P.P., Lin, G., 2018. Contamination of hepatotoxic pyrrolizidine alkaloids in retail honey in China. Food Control 85, 484–494. https://doi.org/10.1016/j.foodcont.2017.10.026.