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The concerning food safety issue of pyrrolizidine alkaloids: An overview

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ABSTRACT

Background: In the last years, several food alerts have identified high levels of pyrrolizidine alkaloids (PAs) in a wide variety of food products, highlighting their occurrence as a concerning food safety issue.
Scope and approach: Currently, there is a call to collect new data on the occurrence of PAs in food matrices that, to date, have been less studied, as well as to develop sensitive and potent analytical methods that enable the determination of these compounds at very low concentration levels, and evaluate the effect of food processing on the stability of these contaminants. Accordingly, this review gives an overview about PAs, regarding general aspects such as their chemical structure, classification, toxicity, risk assessment, occurrence in food, contamination paths and effect of food processing. Likewise, the most relevant analytical procedures for their determination in different food products of the last 10 years (2010–2020) are included.
Key findings and conclusions: PAs exhibit developmental toxicity and have shown to be hepatotoxic, pneumotoxic, genotoxic and carcinogenic. For this reason, it is important to control their occurrence in food through the development of sensitive, selective and environmentally friendly analytical methods that can be properly validated to achieve a correct identification and quantification of these compounds. In the last decade, many efforts have been made to address this food safety issue and maximum concentration limits have been regulated for food products likely to be contaminated with these alkaloids. However, further investigation is required regarding food processing and dilution factors to achieve a reliable assessment of the real intake of these alkaloids by the population and improve the risk management of these natural contaminants.

1. Introduction

In recent years, the number of food alerts reported on the Food and Feed Safety Alerts (RASFF) portal about the high occurrence of pyrrolizidine alkaloids (PAs) and their oxidized forms (pyrrolizidine alkaloids *N*-oxides, PANOS) in different food products has notably increased (Fig. 1a) (RASFF, 2020). As a consequence, the high levels found of these natural toxins (values ranging from 26.5 to 556,910 μg/kg, Table S1) have highlighted their presence as an important food safety issue. PAs are secondary metabolites of plants produced as a defense mechanism against herbivores and insects. To date, more than 600 different structures for PAs (including PANOs) have been described and they have been identified from over 6000 plant species, which mainly belong (about 95%) to the families of *Asteraceae, Fabaceae, Boraginaceae, Orchidaceae and Apocynaceae* (EFSA-European Food Safety Authority, 2011). The intake of PAs has been associated to liver damage, being particularly regarded as one of the major causes of hepatic veno-occlusive disease (HVOD), which can lead to liver cirrhosis and liver failure. Additionally, it can also produce pulmonary hypertension, cardiac hypertrophy, kidneys degenerative injuries or even death (Dusemund et al., 2018; Ma et al., 2018). Moreover, the long-term exposure to these contaminants has been associated to genotoxic and carcinogenicity effects (Dusemund et al., 2018).

The major sources of PAs consumption in humans seem to be plant-derived products contaminated with PA-producing plants. In fact, honey, pollen, teas, herbal teas, food supplements, spices and aromatic herbs are the main food items likely to be contaminated with high levels of PAs/PANOs (Fig. 1b), according to the food alerts notified in the last years (RASFF, 2020). It is believed that the main contamination source of these plant-derived products is due to the accidental co-harvesting of PA-containing weeds (Kaltner, Rychlik, Gareis, & Gottschalk, 2020; Schrenk et al., 2020). However, other contamination paths have been suggested recently, such as the horizontal natural transfer of PAs/PANOs through the soil (Selmar et al., 2019; Selmar, Radwan, & Nowak, 2015) or the intended adulteration by producers for economic benefits (Picron, Herman, Van Hoeck, & Goscinny, 2018a). On the other hand,

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the contamination with PAs has also been detected in products of animal origin, such as milk, meat and eggs (Chen et al., 2021; Chung & Lam, 2018; Diaz, Almeida, & Gardner, 2014; Hoogenboom et al., 2011; Huybrechts & Callebaut, 2015; Mulder et al., 2016, 2018; Yoon et al., 2015), as a consequence of feeding the animals with PA-producing plants. Therefore, due to the widespread occurrence of PAs/PANOs in different types of food and their potential risk for human health, their presence in food should be considered a concerning food safety issue that needs to be addressed. However, to date, further investigation is required, as the real exposure levels of the population to these natural contaminants are still uncertain because of the lack of data. For instance, the evaluation of food processing on the PAs/PANOs content is necessary and useful to provide a real exposure assessment of the population to these compounds. Nevertheless, the effect of food processing on these toxic alkaloids has been scarcely studied, and the limited works available are not conclusive (Cao, Colegate, & Edgar, 2013; Chen, Mulder, Peijnenburg, & Rietjens, 2019; Kempf, Wittig, Schönfeld, et al., 2011; Picron et al., 2018a; Rosemann, 2007, chap. 4). Moreover, it is also necessary to develop sensitive analytical methods enabling the accurate identification and quantification of these compounds at very low concentration levels. As well, it is also important to evaluate their presence in a wide range of food matrices, which have been less studied to date, to broad the knowledge about their occurrence and address this food safety issue (EFSA-European Food Safety Authority, 2016; EFSA-European Food Safety Authority, 2017).

Accordingly, this review aims to give an overview about PAs and their PANOs as a current and concerning food safety issue, regarding general aspects as their chemical structure, classification, toxicity, risk assessment and occurrence in food. As well, the most relevant analytical procedures for their determination in different food products are included, by giving some examples from the last 10 years (from 2010 to 2020). In addition, works regarding the effect of food processing and their different contamination paths are also discussed. Finally, challenges and expected future trends are also included.

2. Chemical structures and toxicity

The common chemical structure in all PAs involves two fused pyrrole rings with a nitrogen heteroatom at position 4, consisting of two structural components: the necine base (amino-alcohol derived from pyrrolizidine) and the necic acid (derived from branched-chain amino acids such as valine, threonine, leucine, or isoleucine) (Fig. 2a). There are two main groups of PAs depending on the existence or not of a double bond between the positions 1 and 2 of the necine base, namely 1,2-unsaturated PAs and saturated PAs (Fig. 2b). Likewise, according to the necine base structure, 1,2-unsaturated PAs can be sorted into: retronecine-, heliotridine- and otonecine-types, while platynecine-type correspond to saturated PAs (Fig. 2c). Retronecine- and heliotridine-types display a bicyclic ring and there are diastereomers among them with different orientation at position 7, whereas otonecine-types are oxidized at position 8 displaying a monocyclic ring (Fig. 2c).

These compounds have been shown to be hepatotoxic, pneumotoxic, genotoxic, carcinogenic and exhibit developmental toxicity (Dusemund et al., 2018). However, PAs themselves are pro-toxins biologically and toxicologically inactive which need to be metabolically activated to exert toxicity. Consequently, not all of them are toxic. In this sense, the presence of the double bond in the necine base increases the toxicity of these compounds, because, once in the body, they are activated into highly reactive pyrrole intermediates, which can lead to cellular adducts and display hepatotoxicity, among other health issues (EFSA-European Food Safety Authority, 2011; Dusemund et al., 2018). In contrast, saturated PAs do not undergo metabolic activation into reactive pyrroles, so they are not considered genotoxic nor carcinogenic (Dusemund et al., 2018). Moreover, depending on the esterification of one or both hydroxyl groups, 1,2-unsaturated PAs can occur as monoesters, open chained diesters or cyclic diesters (Fig. 2d). Additionally, these compounds can also appear as metabolites when they are in their *N*-oxide form or tertiary base (PANOs) (Fig. 2b). However, only retronecine- and heliotridine-type PAs can generate PANOs, as *N*-oxidation is not observed in ontonecine-type PAs because of their methylation in the nitrogen (Moreira, Pereira, Valentão, & Andrade, 2018) (Fig. 2c). Accordingly, based on their structural similarities and botanical origin, 1,2-unsaturated PAs can be classified in four main families (Picron et al., 2018a), which are shown in Table 1.

Several authors have previously reviewed in a more extensive way about the toxicity, metabolism, and risk assessment of pyrrolizidine alkaloids (Dusemund et al., 2018; Xu et al., 2019; Schrenk et al., 2020). In a general way, regarding the toxicokinetics of 1,2-unsaturated PAs, after their oral ingestion they are rapidly absorbed in the gastrointestinal tract. Subsequently, they are metabolically transformed. Their bioactivation mainly takes place in the liver, being this the reason why this organ is the most affected by the toxicity of these compounds. Nevertheless, lungs and kidneys can also be damaged (Moreira et al., 2018). Generally, there are three main pathways for the metabolic activation of PAs: (i) hydrolysis leading to the necine bases and necic acids, (ii) *N*-oxidation of the necine bases to their corresponding PANOs, and (iii) oxidation leading to the generation of highly reactive pyrroles (pyrrolic esters or dehydropyrrolizidine alkaloids (DHPA)) (Fig. 3). The hydrolysis and the *N*-oxidation pathways promote the excretion of PAs and PANOs, whereas the oxidation route is responsible for the high toxicity of these compounds by transforming them into reactive pyrroles (Dusemund et al., 2018; Moreira et al., 2018; Xu et al., 2019). In fact, PANOs can reverse back into PAs and suffer oxidation into toxic DHPA (Fig. 3). The oxidation route is carried out by cytochrome P-450 monooxygenases. The reactive pyrroles generated are the ones responsible for the concerning toxicity of PAs and PANOs. These pyrrolic

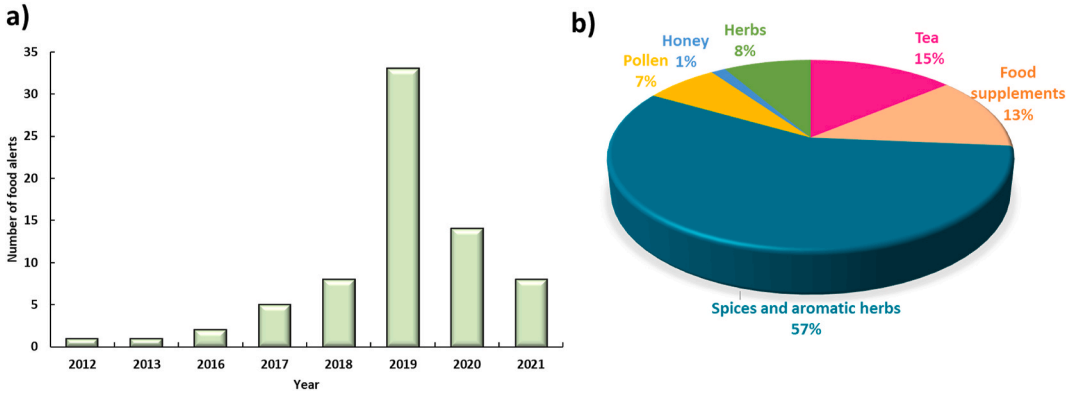

Fig. 1. (a) Evolution of the number of food alerts related to the occurrence of pyrrolizidine alkaloids from 2012 to 2021 and **(b)** distribution of these food alerts according to the food item contaminated (data obtained from RASFF portal, 2021).

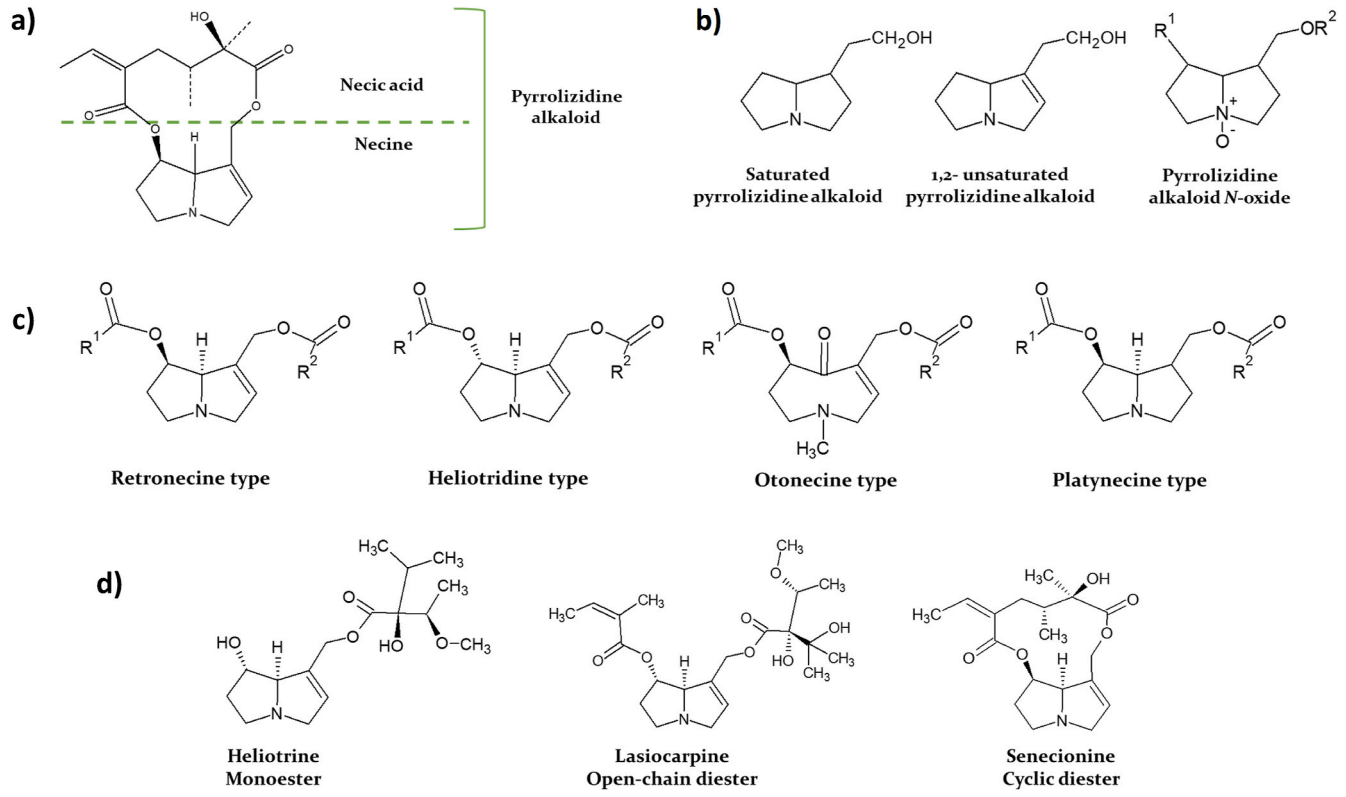

Fig. 2. (a) Common chemical structure of pyrrolizidine alkaloids, **(b)** different forms for pyrrolizidine alkaloids (R^1 and R^2 correspond to different necic acids), **(c)** types of pyrrolizidine alkaloids according to the necine base, and **(d)** different 1,2-pyrrolizidine alkaloids according to their type of esterification.

Table 1
Classification of 1,2-unsaturated pyrrolizidine alkaloids based on their structural similarities and botanical origin.

Family	Chemical structure	Pyrrolizdine alkaloids included	Main botanical origin
Heliotrine-type	Open-chain monoesters and diesters	Heliotrine, europine, lasiocarpine and their *N*-oxides	*Boraginaceae*
Lycopsamine-type	Open-chain monoesters and diesters	Echimidine, indicine, intermedine, lycopsamine and their *N*-oxides	*Boraginaceae, Asteraceae* and *Apocynaceae*
Monocrotaline-type	Macrocyclic diesters	Monocrotaline, monocrotaline *N*-oxide and trichodesmine	*Fabaceae*
Senecionine-type	Macrocyclic diesters	Erucifoline, jacobine, retrorsine, senecionine, seneciphylline, senecivernine, their *N*-oxides and senkirkin	*Asteraceae, Fabaceae* and *Jacobeae*

metabolites act as alkylating agents and can react with enzymes and nucleic acids, inducing acute or chronic hepatotoxicity, genotoxicity and carcinogenicity (Dusemund et al., 2018; Xu et al., 2019) (Fig. 3). They damage the endothelial cells of the centrotubular veins of the liver, causing thickening of their walls and a non-thrombotic obstruction of the hepatic veins, which is known as HVOD, which can lead to cirrhosis and liver failure (Letsyo, Jerz, Winterhalter, & Beuerle, 2017). Likewise, due to their capacity to bind to cellular proteins and DNA, they have also been classified as "possibly carcinogenic to humans" (category 2 B) by the International Agency for Research on Cancer (IARC) (Dusemund et al., 2018).

Although all 1,2-unsaturated PAs share a common metabolic pathway leading to the formation of genotoxic and carcinogenic reactive pyrroles (Fig. 3), different studies have revealed different degrees of toxicity regarding the chemical structure of these compounds. In these sense, cyclic di-esters seem to be markedly the most toxic, followed by open-chain di-esters and finally mono-esters (Merz & Schrenk, 2016; Schrenk et al., 2020). Nevertheless, due to the huge number of PAs and their corresponding PANOs (to date more than 600 different structures have been described) it is impossible to obtain comprehensive *in vivo* data on the toxicity of all congeners. Indeed, there is very limited comparative toxicological data available in the literature for the congeners that are more extensively found as contaminants in food. For this reason, currently, no distinction in the degree of toxicity between PAs is contemplated in risk assessment practice despite their different chemical structure, so all of them are considered equipotent toxic substances with cumulative effects with regard to their carcinogenic activity (EFSA-European Food Safety Authority, 2017). Nonetheless, riddelliine and lasiocarpine are contemplated as the highly toxic PAs. In this sense, several works suggest that some of the PAs that mainly contribute to the dietary exposure levels could be of substantially lower potency than riddelliine or lasiocarpine. However, although this may lead to an overestimation of the risk, the current approach is to consider all of them as potent as these two PAs for the cumulative risk assessment (EFSA-European Food Safety Authority, 2017; Schrenk et al., 2020). Nevertheless, monocrotaline-type PAs/PANOs are not considered relevant in food risk assessments because of very low exposure levels and their low incidence in foodstuffs (Picron, 2018b). Nonetheless, it is worth mentioning that a lot of effort has already gone into defining the relative potency factor for large series of PAs (EFSA-European Food Safety Authority, 2017).

3. Risk management

In Europe, the EFSA (European Food Safety Authority) is the international European body which sets the guidelines to control the occurrence of these compounds in food and feed. Due to the health risk that the intake of these compounds involves mainly because of their potential

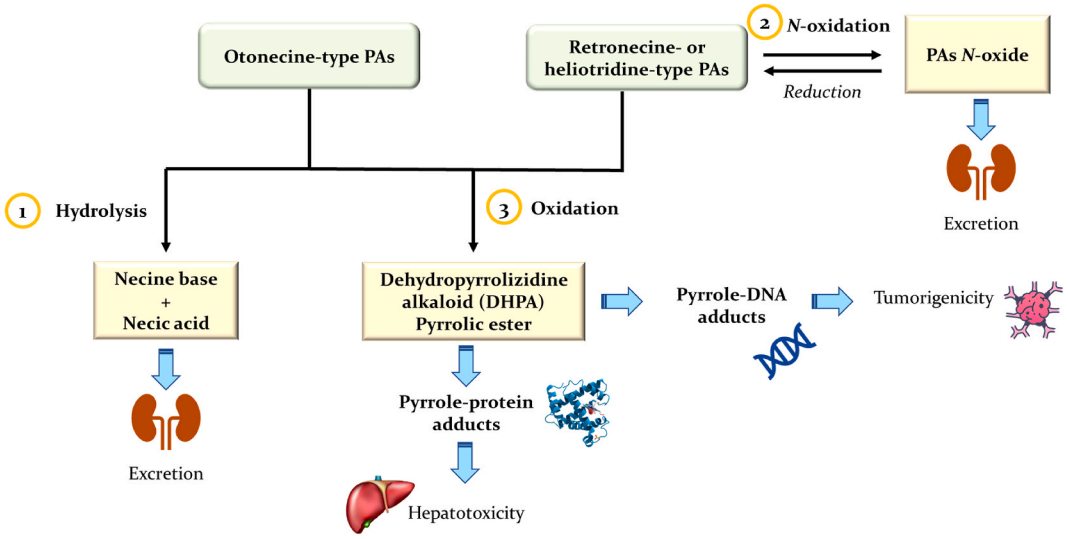

Fig. 3. Main metabolism pathways of pyrrolizidine alkaloids (PAs).

genotoxic and carcinogenic effects, their occurrence in food and feed should be kept as low as reasonably achievable. Between 2007 and 2017, the EFSA published different scientific opinions about the increasing concern of the presence of PAs in food and the need to collect more data about the real exposure through diet (EFSA, 2007; EFSA-European Food Safety Authority, 2011; Mulder, Sánchez, These, Preiss-Weigert, & Castellari, 2015; EFSA-European Food Safety Authority, 2016; EFSA-European Food Safety Authority, 2017). In 2007, a first report was published in which the risk of the presence of PAs in animal feed was evaluated (EFSA, 2007). In this report, a transfer of PAs/PANOs from feed into edible tissues of farm animals was determined, confirming that these alkaloids can be excreted with milk of cows and sheep (although at a low rate), and also in eggs. On the other hand, honey was detected as a foodstuff in which PAs residues are regularly found. Nevertheless, the levels of PAs/PANOs which were found in milk, eggs and honey were significantly lower than the levels found in herbs and spices used for human intake. Afterwards, in 2011, a scientific opinion was issued on PAs in food and feed, focusing on 1,2-unsaturated PAs due to their greater toxicity because of their transformation into reactive pyrroles (EFSA-European Food Safety Authority, 2011), as explained above. In this report, the acute and chronic exposure of PAs by the consumption of honey was estimated for different population groups (toddlers, children and adults). However, due to lack of data, it was not possible to quantify the dietary exposure to these compounds by other foodstuffs. In 2015, the EFSA published an external report that collected data about the occurrence of PAs in different foods, such as milk, dairy products, eggs, meat, meat products and plant-derived products (including herbal teas and food supplements), which were obtained by applying validated analytical methods (Mulder et al., 2015). One year later, in 2016, a report about the exposure of PAs through diet of the European population was published. In this report, the acute and chronic dietary exposure to PAs was estimated through the intake of products of plant origin, mainly tea, herbal teas and honey (EFSA-European Food Safety Authority, 2016). Finally, in 2017, the EFSA published a report which evaluated the human health risks derived from the presence of PAs in honey, tea, herbal teas and food supplements (EFSA-European Food Safety Authority, 2017). From all these reports and documents, among other recommendations, it can be concluded that the exposure levels of the population to PAs and PANOs are still uncertain. Therefore, there is a need to keep collecting data about the occurrence of PAs in different foods, so that the uncertainty of exposure to these contaminants can be reduced. Recently, maximum concentration levels of these alkaloids in some food items have been legislated (Table 2) by Commission Regulation (EU) 2020/2040 amending Regulation (EC) No. 1881/2006. These limits refer to the maximum total concentration of PAs/PANOs that can be found in different foods, which range from 1.0 to 1000 μg/kg (Table 2). In addition, at this moment, the EFSA recommends a set of 17 PAs/PANOs which must be monitored in food items, including intermedine, intermedine-*N*-oxide, lycopsamine, lycopsamine-*N*-oxide, senecionine, senecionine-*N*-oxide, senecivernine, senecivernine-*N*-oxide, seneciphylline, seneciphylline-*N*-oxide, retrorsine, retrorsine-*N*-oxide, echimidine, echimidine-*N*-oxide, lasiocarpine, lasiocarpine-*N*-oxide and senkirkine (EFSA-European Food Safety Authority, 2017). These compounds have been selected due to their concerning toxicity and their frequent occurrence in food. However, it is

Table 2
Maximum concentration levels for pyrrolizidine alkaloids in different food products (data obtained from Commission Regulation (EU) 2020/2040).

Food Product	Maximum concentration level proposed (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 with the exception of pollen based food supplements, pollen an 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.

currently being considered to increase the number of PAs/PANOs monitored in food from 17 to 21 (Fig. 4), by including europine, heliotrine and their respective *N*-oxides, due to the notable occurrence of these compounds in some foods (Picron, 2018b; Picron et al., 2018a).

On the other hand, other specific institutions in Europe have also set guidelines to monitor the presence of these contaminants. In this sense, in Germany PAs have been regulated since 1992 by a Federal Pharmaceutical Ordinance (Bundesgesundheitsamt, 1992). According to this regulation, the total oral intake limit of 1,2-unsaturated PAs (including PANOs) must not exceed from 1 μg PA/PANOS per day, but if the intake is longer than 6 weeks the limit is reduced to 0.1 μg PA/PANOS per day. However, in 2007, a multidisciplinary committee of the German Federal Institute for Risk Assessment (Bundesinstitut für Risikobewertung, BfR) considered to apply the "zero tolerance principle" proposed by the European Union when the risk cannot be calculated, the limits of exposure cannot be derived due to the lack of scientific data or the compounds present carcinogenic or mutagenic effects (BfR, 2007). Consequently, in 2011 it was recommended to keep the total exposure to PAs as low as possible, not exceeding from a daily intake of 0.007 μg/kg body weight (BfR, 2011). Since then, the BfR recommends keeping the total exposure of consumers to PAs as low as possible and to check the PAs content in the batches of different food sources (such as herbal teas) before their distribution into the market. In Switzerland, the same regulations for phytopharmaceuticals are applied as in Germany, and in Austria the legal situation for herbal remedies is similar (Bundesgesetzblatt, 1989; Merz & Schrenk, 2016). Only a few PA-producing plants and their preparations are authorized in Austria and can only be marketed after being analyzed by a reliable detection method which proves that the final product does not contain PAs (Bundesgesetzblatt, 1989). Similarly, in Belgium, the only PA-producing plant which is banned for consumption is borage, and its oil can only be used for food supplements after being analyzed with a suitable detection method and showing to be free of PAs (Koninklijk besluit, 1997). On the other hand, in the Netherlands, the limit of PAs is set in 1 μg/kg for herbal solid preparations and in 1 μg/L for herbal liquid extracts, whereas a tolerable daily intake of 0.1 μg/kg body weight for non-cancer effects has been proposed by the Dutch National Institute for Public Health and the Environment (RIVM) (Kräuterbeschluss, 2001; RIVM, 2005; RIVM, 2007). In the case of United Kingdom (UK), comfrey and its preparations are banned, and the UK Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) established that PAs doses up to 0.007 μg/kg body weight per day are unlikely to promote cancer risk. Nevertheless, the COT suggests a maximum PAs limit of 6.4 μg/kg for honey (COT, 2008). The European Medicines Agency also recommends a maximum daily intake of 0.007 μg/kg body weight (EMA-European Medicines Agency, 2016).

In other non-European regions, such as Australia and New Zealand, the human consumption of PAs is only considered a risk in a chronic exposure scenario. Thus, a tolerable daily intake of 1 μg/kg body weight is recommended in these countries (ANZFA, 2001). Moreover, the Food Standards Australia New Zealand (FSANZ) encourage honey producers to mix highly contaminated honeys with PAs (those mainly derived from *E. plantagineum*) with honeys free of PAs to not exceed the limits established (FSANZ, 2004). This measure collides with the EFSA directive of non-dilution of contaminated food and feedstuff (EFSA, 2007), and seems more reasonable to withdraw contaminated honeys from the market instead of contaminating others free of PAs, so the overall presence of PAs in the human food chain can be reduced. In contrast, the US Food and Drug Administration (FDA) has banned the marketing of any product containing PAs (FDA, 2001), but as in the case of the EFSA in Europe, it has not been possible to establish a safe oral exposure due to the scarcity of available data. Therefore, as drawn from the different risk assessment authorities, it can be concluded that, to date, there is no consensus in the safe oral exposure limit of PAs.

4. Occurrence in food products and contamination paths

The most relevant works within the last 10 years in which PAs/PANOs are determined in different food products through different analytical strategies are gathered in Table 3. As it can be observed, the occurrence of PAs/PANOs has been evaluated in both plant- and animal-derived products including honey, cereals, flours, salads, teas, herbal teas, spices, aromatic herbs, milk and dairy products, eggs, meat and meat products, as well as food supplements, beverages and snacks. To date, it has been revealed that is not frequent to find contamination of PAs in animal-derived products. Nonetheless, if these type of products show contamination with PAs, the concentration levels found of these contaminants will be low. PA-containing plants may be present in forage fields were animals feed or in fresh or dry products widely use as feed products. Thus, these contaminants can be transferred to animal-derived

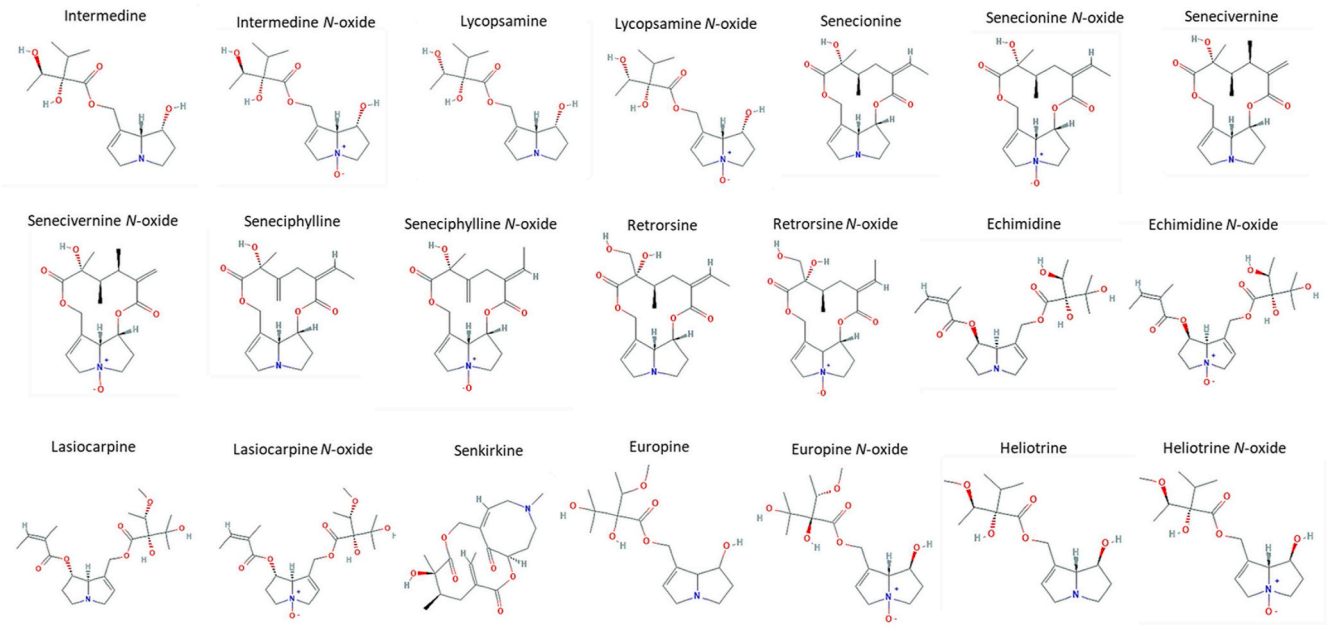

Fig. 4. Pyrrolizidine alkaloids recommended by the European Food Safety Authority to be monitored in food items.

Table 3
Analytical methods for the determination of pyrrolizidine alkaloids and their concentration levels found in food samples (2010–2020).

Food matrix	Number of PAs/ PANOs	Sample preparation	Analysis	LOD/LOQ	Recoveries (%)	Range of PAs content found	Ref.
Milk	21	Precipitation with MeOH containing 0.1% formic acid and concentration by evaporation.	UHPLC-QHQ-MS/MS MRM mode Column: C18 at 50 °C	-/0.05–0.2 μg/L	44–67%	9.71 μg/L	Hoogenboom et al. (2011)
Honey	17	LLE with H_2SO_4 (0.05 M) followed by SCX-SPE.	HPLC-QTRAP-MS/MS Positive ion mode and MRM mode Column: C18 at 25 °C	-/1–3 μg/kg	60–110%	1–1087 μg/kg	Dübecke, Beckh, and Lüllmann (2011)
Honey	16	*For HPLC-MS/MS analysis*: Dilution with water followed by QuEChERS procedure and reversed phase online-SPE. *For GC-MS*: LLE with H_2SO_4 (0.05 M) and addition of zinc, followed by SCX-SPE. The extract was reduced with 1 M $LiAlH_4$ solution in THF, followed by the addition of dichloromethane and 10% NaOH and purification of the organic phase by SPE with sodium sulfate covered with celite. Finally, derivatization with MSTFA.	HPLC-TQ-MS/MS ESI positive ion mode Column: C18 HRGC-Q-MS EI and SIM mode Column: DB-1MS (Polysiloxane) fused-silica capillary column	-/1–50 μg/kg (HPLC-MS/MS) -/10 μg/kg (GC-MS)	97–105%	0–13019 μg/kg (HPLC-MS/MS)	Kempf, Wittig, Reinhard, et al. (2011)
Honey, pollen and honey-based products (mead, candy, fennel honey, soft drinks, power bars, cereals, jelly babies, baby food, supplements, fruit sauce)	6	*Mead and fennel honey*: LLE with H_2SO_4 (0.05 M), addition of zinc and purification by SCX-SPE. The extract was reduced with 1 M $LiAlH_4$ solution in THF, followed by the addition of dichloromethane and 10% NaOH and purification of the organic phase by SPE with sodium sulfate covered with celite. Finally, derivatization with MSTFA. *Other foodstuff*: dilution with water and acidification with 0.5 M H_2SO_4, alkalization with 10% NaOH to pH 11.0, LLE with pentane: dichloromethane (2:1, v/v) followed by SCX-SPE. The extract was subjected to the same procedure as the mead and fennel honeys.	HRGC-Q-MS EI and SIM mode Column: DB-1MS (Polysiloxane) fused-silica capillary column	-/10 μg/kg	74–88%	0.010–0.484 mg/g	Kempf, Wittig, Schönfeld, et al. (2011)
Honey	2	Addition of zinc, filtration through glass wool followed by SCX-SPE. The extract was evaporated to dryness and reconstituted in MeOH. Addition of 1 M $LiAlH_4$ solution in THF, followed by the addition of dichloromethane, 10% NaOH. Purification with sodium sulfate and glass wool. Finally, derivatization with MSTFA.	HRGC-Q-MS SIM mode Column: ZB-5MS (Arylene polymer) capillary column	2.0/6.0 μg retronecine equivalents/Kg	–	10.6–494.5 μg/kg	Cramer et al. (2012)
Honey and mead	7	*Honey*: LLE with MeOH at 40 °C followed by SCX-SPE. *Mead*: pH adjusted to 1.6–2.7 with H_2SO_4 (0.05 M) followed by SCX-SPE.	HPLC-IT-MS/MS ESI positive ion mode Column: C18	50/- ng/g	–	Honey: 780 μg/kg Mead: 236–540 μg/L	Cao et al. (2013)
Honey	11	LLE with H_2SO_4 (0.05 M) at 40 °C followed by SCX-SPE.	HPLC-IT-MS/MS ESI positive ion mode Column: C18 at 30 °C	0.0134 - 0.0305/ 0.0446–0.1018 μg/mL	87%	182–5614 μg/kg	Griffin, Danaher, Elliott, Kennedy, and Furey (2013)
Honey and culinary herbs	3	LLE or SLE with H_2SO_4 (0.05 M) and purification by SCX-SPE. The extract was reduced with 1 M $LiAlH_4$ solution in THF, followed by the addition of dichloromethane and 10% NaOH and purification of the organic phase by SPE with sodium sulfate and glass wool. Finally, derivatization with phthalic anhydride in pyridine.	HPLC-QTRAP-MS/MS ESI positive ion mode and MRM mode Column: silica with Pentafluorophenylpropyl	0.1–1.0/0.3–3.0 μg retronecine equivalents/Kg	69–104%	0.9–74 μg/kg	Cramer et al. (2013)
Honey	17	LLE with H_2SO_4 (0.5 M) at 37 °C followed by SCX-SPE.	HPLC-TQ-MS/MS ESI positive ion mode and MRM	-/1–3 μg/kg	>80%	1–237 μg/kg	Orantes-Bermejo et al. (2013)

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Table 3 (*continued*)

Food matrix	Number of PAs/ PANOs	Sample preparation	Analysis	LOD/LOQ	Recoveries (%)	Range of PAs content found	Ref.
			mode Column: C18				
Eggs	2	SLE with H_2SO_4 (0.05 M) and ACN, followed by SCX-SPE.	HPLC-IT-MS/MS ESI positive ion mode Column: polar-reversed phase	-/2 ng/g	–	9.5–885 μg/kg	Diaz et al. (2014)
Herbal dietary supplements	11	QuEChERS.	UHPLC-Q-Orbitrap-MS/MS ESI positive ion mode and HRMS mode Column: HSS T3 at 40 °C	≤10/≤50–2500 μg/kg	70–120%	319 μg/kg	Vaclavik et al. (2014)
Honey	9	LLE with H_2SO_4 (0.05 M), addition of zinc followed by QuEChERS.	UHPLC-Q-MS ESI positive ion mode and SIM mode Column: C8 at 34 °C	0.021–1.39/ 0.081–4.35 μg/kg	67–122%	1–172 μg/kg	Martinello et al. (2014)
Herbal teas	14	SLE with H_2SO_4 (0.05 M) at 40 °C followed by SCX-SPE.	HPLC-TQ-MS/MS ESI positive ion mode and MRM mode Column: PFP at 35 °C	0.4 - 1.9/1.3–6.3 μg/kg	93–127%	10–1733 μg/kg	Griffin, Gosetto, Danaher, Sabatini, and Furey (2014)
Honey and (herbal) teas	17	*Honey samples*: LLE with H_2SO_4 (0.05 M) at 40 °C followed by SCX-SPE. *Tea samples*: SLE with H_2SO_4 (0.05 M), pH adjusted to 6.0–7.0 with ammonia solution followed by C18-SPE.	HPLC-TQ-MS/MS ESI positive ion mode and MRM mode Column: C18	0.06–2.0/0.18–6.4 μg/kg	45–122	0.3–5647 μg/kg	Bodi et al. (2014)
Honey	18	LLE with H_2SO_4 (0.05 M) followed by SCX-SPE.	HPLC-QTRAP-MS/MS Positive ion mode and MRM mode Column: C18 at 25 °C	-/1–3 μg/kg	–	0.4–55 μg/kg	Kast et al. (2014)
Soybean, seed oils, milks, and margarines	9	*Soybeans and milk*: SLE or LLE with chloroform: MeOH (1:1, v/v), evaporation of the extract to dryness and reconstitution in MeOH, lipid precipitation at −24 °C, followed by SCX-SPE. *Margarine and seed oils*: SLE or LLE with MeOH, lipid precipitation at −24 °C, followed by SCX-SPE.	HPLC-TQ-MS/MS ESI positive ion mode and MRM mode Column: C18 at 30 °C	0.07–0.59/ 0.20–1.43 ng/mL	82–105%	0.64–26.96 μg/kg	Yoon et al. (2015)
Milk, honey, (herbal) tea	16	*Milk samples*: LLE with HCl and hexane at 60 °C. *Honey samples*: LLE with HCl solution containing 20% of NaCl at 60 °C. *Solid samples*: SLE with HCl solution containing 20% of NaCl and hexane.	UHPLC-TQ-MS/MS ESI positive ion mode and MRM mode Column: C18 at 40 °C	0.001–0.22/ 0.003–0.73 ng/g	80–114%	0.011–314.23 μg/kg	Huybrechts et al. (2015)
Leek, wheat, and tea	11	QuEChERS.	HPLC-Q-Orbitrap-MS/MS ESI both positive and negative ion mode and HRMS mode Column: polar-reversed phase at 25 °C	-/≤1–100 μg/kg	71–93%	–	Dzuman et al. (2015)
Honey	14	LLE with H_2SO_4 (0.05 mol/L) followed by SCX-SPE.	HPLC-TQ-MS/MS ESI positive ion mode and MRM mode Column: PFP at 35 °C	0.4 - 3.3/1.4–10.9 μg/kg	82–112%	3–932 μg/kg	Griffin, Mitrovic, Danaher, and Furey (2015)
Honey	14	LLE with H_2SO_4 (0.05 M) followed by SCX-SPE.	HPLC-TQ-MS/MS Positive ion mode and MRM mode Column: C18 at 30 °C	0.5 - 3.9/2.3–12.9 μg/kg	70–125%	2.9–545.5 μg/kg.	Griffin, O'Mahony, Danaher, and Furey (2015)
Teas and herbal teas	28	SLE with aqueous acetic acid:MeOH solution (1:2, v/v) and pH adjusted to 5.0–6.0 with diluted ammonia solution.	HPLC-QTRAP-MS/MS MRM mode Column: C18 at 25 °C	-/10–50 μg/kg	80–95%	20–1729 μg/kg	Shimshoni, Duebecke, Mulder, Cuneah, and Barel (2015)
Honey	5	LLE with H_2SO_4 (0.05 M) followed by SCX-SPE.			40–106%	2.4–2.7 μg/kg	Mudge et al. (2015)

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Table 3 (*continued*)

Food matrix	Number of PAs/ PANOs	Sample preparation	Analysis	LOD/LOQ	Recoveries (%)	Range of PAs content found	Ref.
			HPLC-TQ-MS/MS ESI positive ion mode and SIM mode Column: C18 at 25 °C	0.45–0.67/ 1.21–1.79 ng/mL			
Herbal teas	23	*Dry samples*: SLE with H_2SO_4 (0.05 M), pH adjusted to 6.0–7.0 with ammonia solution followed by C18-SPE. *Infusion samples*: infusion with boiling water followed by C18-SPE.	HPLC-TQ-MS/MS ESI positive ion mode and MRM mode Column: C18 at 20 °C	-/10 μg/kg	76–125%	0–5668 μg/kg	Schulz et al. (2015)
Herbal food supplements	25	SLE with MeOH.	UHPLC-QToF-MS/MS ESI positive ion mode and All-ion MS/MS mode Column: C18 at 40 °C	0.05–5/- ng/mL	–	101–8400 ng/g	Avula et al. (2015)
Eggs and meat	51	SLE with 0.2% formic acid solution and hexane. The aqueous phase was adjusted to pH 9.0–10.0 with ammonia solution followed by reversed phase SPE.	UHPLC-TQ-MS/MS ESI positive ion mode and MRM mode Column: C18 at 50 °C	-/0.1–1 μg/kg	–	0.30–392 μg/kg	Mulder et al. (2016)
Honey	8	Dilution with deionized wáter.	HPLC-QTRAP-MS/MS ESI positive ion mode and MRM mode Column: C18	0.1–1.0/0.2–1.5 μg/ kg	93–110%	1.2–248 μg/kg	Valese et al. (2016)
Honey	9	LLE with H_2SO_4 (0.1 M), addition of zinc followed by QuEChERS.	HPLC-Q-Orbitrap-MS/MS ESI positive ion mode and HRMS mode Column: C8 at 35 °C	0.04 - 0.2/0.1–0.7 μg/kg	92–115%	–	Martinello et al. (2017)
Milk, dairy products, eggs, meat, meat products, (herbal) teas and (herbal) food supplements	38	*Animal-derived samples*: LLE or SLE with 0.2% aqueous formic acid solution and hexane. The aqueous phase was adjusted to pH 9.0–10.0 with ammonia solution followed by reversed phase SPE. *(herbal) tea samples*: infusion with boiling water followed by C18-SPE. *Food supplements*: SLE with H_2SO_4 (0.05 M), pH adjusted to 6.0–7.0 with ammonia solution followed by C18-SPE. *Oily food supplements*: SLE with H_2SO_4 (0.05 M) followed by SCX-SPE.	UHPLC-TQ-MS/MS ESI positive ion mode and MRM mode Column: C18 at 50 °C	0.03–0.05/- μg/L (milk and yoghurt) 0.05–0.15/- μg/Kg (egg, cheese, chicken and pork meat) 0.1–0.25/- μg/Kg (beef and liver) 0.2–3.8/- μg/Kg (teas and food supplements)	30–122%	0.05–0.16 μg/L 0.10–2410275 μg/kg	Mulder et al., (2018)
Cereal products, dairy products, meat, eggs, honey, tea infusion, and spices.	28	SLE or LLE with H_2SO_4 (0.05 M), pH adjusted to 6.0–7.0 with ammonia solution followed by C18-SPE.	UHPLC-QTRAP-MS/MS ESI positive ion mode and MRM mode Column: C18 at 40 °C	-/0.010–0.76 μg/kg	50–120%	–	Chung et al. (2018)
Salads, herbs, spices, tea, herbal teas, tea infusions and ice-tea beverages	31	*Dry plant samples*: SLE with MeOH containing 0.1% formic acid followed by SPE with graphitized non-porous carbon as sorbent. *Infusion extracts*: infusion of herbal teas with boiling water, basification to pH 9.0–10.0 with 28–30% ammonia followed by C18-SPE. *Ice-tea beverages*: basification to pH 9.0–10.0 followed by C18-SPE.	UHPLC-TQ-MS/MS ESI positive ion mode and MRM mode Column: C18 at 45 °C	-/0.1–1 ng/g (plant extracts) -/0.01 ng/mL (infusion extracts)	86–125%	0.01–187151 μg/ kg 0.01–2106 μg/L	Picron et al. (2018a)
Peppermint tea and honey	25	SLE or LLE with H_2SO_4 (0.05 M) followed by SCX-SPE.	HPLC-TQ-MS/MS ESI positive ion mode and MRM mode Column: polar-reversed phase at 30 °C	0.01–1.60/ 0.03–5.40 μg/kg	49–121%	–	Kaltner et al. (2018)
Honey	4 (sum of all 1, 2-unsaturated	LLE with HCl (0.15 M), addition of zinc, filtration through cellulose filters, followed by SPE mixed-	GC-Q-MS EI mode	-/1 μg/kg	73–94%	1.0–64.1 μg/kg	Kowalczyk et al. (2018)

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Table 3 (*continued*)

Food matrix	Number of PAs/ PANOs	Sample preparation	Analysis	LOD/LOQ	Recoveries (%)	Range of PAs content found	Ref.
	retronecine/ heliotridine-type PAs)	mode (reversed phase and SCX). Extract evaporated to dryness and reconstituted in ethyl acetate and MeOH and reduced with 1 M $LiAlH_4$ solution in THF, followed by the addition of dichloromethane and 10% NaOH and purification of the organic phase with sodium sulfate. Finally, derivatization with ethyl acetate anhydrous and HFBA.	Column: DB-5 MS (nonpolar phenyl arylene polymer) capillary column				
Herbal teas	70	Infusion with boiling water.	UHPLC-TQ-MS/MS MRM mode Column: C18 at 50 °C	0.01 - 0.02/0.05 μg/L	73–107%	30.7–1120 μg/L	Chen et al. (2019)
Honey	12	LLE with H_2SO_4 (0.05 M) followed by SPE mixed-mode (reversed phase and SCX).	HPLC-QToF-MS/MS ESI positive ion mode and HRMS mode Column: C18 at 40 °C	0.2–0.6/0.5–1.3 μg/ kg	79–104%	1.4–14.2 μg/kg	Wang et al. (2019)
Teas and herbal teas	44	SLE with H_2SO_4 (0.05 M) followed by SCX-SPE.	HPLC-TQ-MS/MS ESI positive ion mode and MRM mode Column: C18 at 30 °C	0.1–7.0/0.1–27.9 μg/ kg	52–152%	0.1–47.9 μg/g	Kaltner et al., (2019)
Herbs	12	SLE with 2% formic acid.	HPLC-TQ-MS/MS ESI positive ion mode and MRM mode Column: C18	–	–	11–1777 μg/kg	Selmar et al. (2019)
Liqueurs, elixirs and herbal juices	30	LLE with H_2SO_4 (0.05 M) and purification by SCX-SPE. The extract was reduced with 1 M $LiAlH_4$ solution in THF, followed by the addition of dichloromethane and 10% NaOH and purification of the organic phase by SPE with sodium sulfate and glass wool. Finally, derivatization with phthalic anhydride in pyridine.	HPLC-QTRAP-MS/MS Positive ion mode and MRM mode Column: C18 at 25 °C	–	–	0.21–3121 μg/kg	Chmit et al. (2019)
Honey	9	Dilution with acidified water (0.25 M acetic acid), addition of zinc and pH adjusted to 9.5. Subsequently DLLME (dispersive liquid-liquid microextraction) with chloroform and isopropyl alcohol.	UHPLC-QTRAP-MS/MS Positive ion mode and MRM mode Column: Polar C18 at 30 °C	-/0.03–0.06 μg/kg	63–103%	0.2–17.5 μg/kg	Celano et al. (2019)
Spices and culinary herbs	44	SLE with H_2SO_4 (0.05 M) followed by SCX-SPE.	HPLC-TQ-MS/MS ESI positive ion mode and MRM mode Column: C18 at 30 °C	<0.1–2.6/- μg/Kg	50–119% (for 40 of 44 analytes)	0.1–24600 μg/kg	Kaltner et al. (2020)
Oregano	21	μ-QuEChERS.	UHPLC-IT-MS/MS ESI positive ion mode and MRM mode Column: Polar C18 at 25 °C	0.1–7.5/0.5–25.0 μg/ kg	77–96%	334–6375 μg/kg	Izcara et al. (2020)
Herbs	30	QuEChERS.	HPLC-QTRAP-MS/MS Positive ion mode and MRM mode Column: C18 at 40 °C	-/1 μg/kg	61–128%	8–41 μg/kg	Kaczyński and Łozowicka (2020)
Maize	1 (Sum of all 1, 2-unsaturated retronecine/ heliotridine-type PAs)	SLE with H_2SO_4 (0.05 M), filtration through glass wool followed by SCX-SPE. Extract evaporated to dryness and reconstituted in MeOH and reduced with 1 M $LiAlH_4$ solution in THF, followed by the addition of dichloromethane and 10% NaOH and purification of the organic phase with sodium sulfate and glass wool. Finally, derivatization with phthalic anhydride in pyridine.	HPLC-QTRAP-MS/MS ESI positive ion mode and MRM mode Column: C12 at 40 °C	–	–	0.9–6.6 μg/kg	Letsyo et al. (2020)

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Table 3 (*continued*)

Food matrix	Number of PAs/ PANOs	Sample preparation	Analysis	LOD/LOQ	Recoveries (%)	Range of PAs content found	Ref.
Honey	17	LLE with 0.05 M H_2SO_4:MeOH (85:15, v/v) followed by SPE mixed-mode (reversed phase and SCX).	HPLC-TQ-MS/MS Positive ion mode and MRM mode Column: C18	–	–	0.2–281.1 mg/kg	He et al. (2020)
Honey	2	LLE with H_2SO_4 (0.05 M) followed by SCX-SPE.	HPLC-DAD λ: 223 nm Column: C18	–	–	–	Moreira et al. (2020)
Milk	6	LLE with 0.5% formic acid followed by LLE with dichloromethane.	DART-IT-MS Positive ion mode	0.55 - 0.85/ 1.83–2.82 ng/mL	89–112%	2.02–355 μg/g	Chen et al. (2021)

C8: octyl bonded silica; C12: dodecyl bonded silica; C18: octadecyl bonded silica; DAD: Diode array detection; DART: Direct analysis in real time; DLLME: Dispersive liquid-liquid microextraction; EI: Electron ionization; ESI: electrospray ionization; GC: Gas chromatography; HFBA: heptafluorobutyric anhydride; HPLC: High performance liquid chromatography; HRGC: High resolution gas chromatography; HRMS: high resolution mass spectrometry; IT: Ion-trap; LLE: Liquid-Liquid extraction; LOD: limit of detection; LOQ: limit of quantification; MeOH: methanol; MRM: multiple reaction monitoring; MS: Mass spectrometry; MS/MS: tandem mass spectrometry; MSTFA: *N*-Methyl-*N*-(trimethylsilyl)trifluoroacetamide; PANOs: pyrrolizidine alkaloikds *N*-oxide; PAs: pyrrolizidine alkaloikds; PFP: Pentafluorophenyl Propyl; Q: Single quadrupole; QHQ: quadrupole-hexapole-quadrupole analyzer; QToF: Quadrupole time-of-flight; QTRAP: hybrid triple quadrupole-linear ion trap; QuEChERS: Quick, easy, cheap, effective, rugged and safe; SCX: Strong cation exchange; SIM: Selected ion monitoring; SLE: Solid-Liquid extraction; SPE: solid-phase extraction; THF: Tetrahydrofuran; TQ: Triple quadrupole; UHPLC: Ultra-high performance liquid chromatography.

products when they are consumed by animals (EFSA-European Food Safety Authority, 2011). In fact, different transfer experiments of PAs to eggs, meat and milk have confirmed this phenomenon (Edgar & Smith, 2000; Colegate, Boppré, & Molyneux, 2011; Hoogenboom et al., 2011; Diaz et al., 2014; Mulder et al., 2016; Edgar). Nevertheless, in the external report published by the EFSA in 2015 (Mulder et al., 2015) it was concluded that the natural occurrence of PAs contamination of eggs and meat products seemed to be rare in the European Union, whereas it was more frequent in milk, although at very low concentration levels. Indeed, in this work it was revealed that *Senecio* spp. and species from the *Boraginaceae* family could be responsible for the presence of PAs in the positive milk samples contaminated (Mulder et al., 2015). More recently, in 2018, Mulder et al. (2018) carried out an analysis in the European market that revealed that the 6% of the milk samples (11 out of 182) and a 1% of the egg samples (2 out of 205) analyzed were contaminated with PAs. Nonetheless, the levels of PAs found were relatively very low, and no PAs were detected in the meat and meat products analyzed. On the other hand, in a Belgian market survey the occurrence of PAs was detected, besides from milk and dairy products, also in meat products, being liver products the most contaminated among the meat samples analyzed and, also, products based on duck meat (Picron, 2019).

Conversely, PAs have been extensively detected in plant-derived products. Accordingly, the EFSA has identified honey, teas, herbal teas and food supplements as the main food items likely to be contaminated with high levels of PAs/PANOs (EFSA-European Food Safety Authority, 2017). In fact, in a recent study, it was stated that the 91% of the (herbal) teas and the 60% of the food supplements analyzed contained measurable amounts of at least one individual PAs (Mulder, 2018). In fact, since 2012, the food alerts reported in the RASFF portal related to the presence of PAs/PANOs in food products were mainly raised for herbal food supplements (mainly those containing St John's wort (*Hypericum perforatum*) and borage), pollen, honey and (herbal) teas (RASFF, 2020) (Fig. 1b). For this reason, many authors have addressed the presence of PAs in these food matrices (Table 3). However, in the last two years, these alerts have noticeably increased for other products such as spices and aromatic herbs (Fig. 1b), highlighting the striking number of alerts raised for the relatively high amounts of PAs found in oregano (ranging from 6660 to 133870 μg/kg) (RASFF, 2020). Nonetheless, currently, works focusing on the detection of PAs/PANOs in spices and aromatic herbs are scarcer in the literature than for other food matrices (Cramer, Schiebel, Ernst, & Beuerle, 2013; Izcara, Casado, Morante-Zarcero, & Sierra, 2020; Kaltner et al., 2020; Kapp, 2017; Picron et al., 2018a). Table S1 shows a detailed description of the serious food alerts issued since 2012 related to the occurrence of PAs/PANOs in different food products.

It was first widely assumed that the contamination of these plant-derived commodities with PAs/PANOs was due to the accidental inclusion of weeds or impurities from PA-producing plants during harvest (Kaltner et al., 2020). Nevertheless, alternative contamination paths involving horizontal natural transfer of PAs/PANOs through the soil have also been suggested recently in several works (Nowak et al., 2016; Selmar et al., 2015, 2019). In this sense, PAs can be leached out from dead and decomposing plant materials into the soil leading to their uptake by acceptor plants (non PA-producing plants) (Selmar et al., 2015, 2019). Preliminary studies by mulching plants free of PAs (peppermint and camomile) with dry *Senecio* leaves (a PA-producing plant) confirmed the uptake of PAs via the soil (Selmar et al., 2015). Nevertheless, it has been confirmed that this horizontal natural transfer through the soil can also happen among living plants growing nearby (Selmar et al., 2019). Accordingly, Selmar et al. (2019) co-cultivated ragwort (*Senecio jacobaea)*, a PA-producing plant, with parsley (a plant free of PAs) in the same pot. It was observed that the PAs synthesized by the *Senecio* donor plant were translocated into the parsley plant. To evaluate if the PAs transfer could happen by direct contact of the leaves of both plants, an additional experiment using plastic

enclosures to prevent this contact among leaves was performed. The amount of PAs transferred into the acceptor plant was similar with and without the plastic enclosures, so a direct leaf to leaf transfer was discarded (Selmar et al., 2019). Moreover, in the same work, co-cultivations under field conditions of ragwort with acceptor plants free of PAs (*Petroselinum crispum, Melissa officinalis, Matricaria recutita, Mentha piperita* and *Tropaeolum majus*) were also carried out to evaluate the transfer of PAs among living plants growing in the vicinity. In all cases, PAs were present in these acceptor plants after co-cultivation with ragwort (Selmar et al., 2019). With these experiments, it was confirmed that the transfer of PAs from donor to acceptor plants involves an uptake through the soil, ruling out a direct leaf to leaf or root to root transfer. This horizontal natural transfer of PAs/PANOs through the soil has also been suggested in a recent work for a wild oregano sample (Izcara et al., 2020). Oregano is a *Lamiaceae* plant (a family of non-PA-producing plants). Thus, the occurrence of PAs/PANOs was not expected in a branch sample obtained from a wild crop field of oregano. However, when the sample was analyzed it was surprisingly contaminated with a significant amount of these alkaloids (928 μg/kg) (Izcara et al., 2020). Therefore, these results reinforce the horizontal natural transfer theory through soil as a path of contamination, so the occurrence of these contaminants in plant-derived products should not only be considered as a result of the accidental inclusion of PA-producing foreign plants during harvest (cross-contamination) or to their intended adulteration, as it has been suggested in other works (Black, Haughey, Chevallier, Galvin-King, & Elliott, 2016; Kaltner et al., 2020; Kapp, Hägele, & Plate, 2019; Picron et al., 2018a; Picron, Herman, Van Hoeck, & Goscinny, 2020).

On the other hand, in the case of honey samples, their contamination with PAs/PANOs is in general accidently produced via pollen dislodge into nectar by the bees collecting the pollen and the nectar from PA-producing plants (Kempf, Wittig, Schönfeld, et al., 2011). Nonetheless, beekeepers can also accidently or deliberately introduce pollen into honey during its production (Edgar, Colegate, Boppré, & Molyneux, 2011; Ma et al., 2018). In fact, in many countries beekeepers regularly use some PA-producing plants in the production of honey (Edgar et al., 2011; Moreira et al., 2018). Thus, a preventive measure, which could help to reduce the contamination of honey with PAs/PANOs, could be the introduction of good beekeeping practices, such as selecting carefully the locations to place the bee hives and learn about the PA-producing plants that may be attractive for bees. In this sense, it has been stated that the main plants responsible for the occurrence of these alkaloids in honey samples are *Echium* spp., *Senecio* spp., *Eupatorium* spp. and *Borago* spp. (Kempf, Reinhard, & Beuerle, 2010; Moreira et al., 2018). Consequently, senecionine, echimidine and lycopsamine are the PAs most commonly found in beehive products and derivatives (Moreira et al., 2018; Picron et al., 2020). Nonetheless, the occurrence of these alkaloids is also directly influenced by the geographical origin. For instance, in a Belgian market survey carried out by Picron et al. (2020), the contamination of PAs was evaluated in both foreign and Belgian honey samples. The 90% of the foreign samples analyzed were contaminated and belonged to Mediterranean countries like Spain, France, Greece and Turkey. Also, honeys originating in Latin America were included in the work. In these samples, it was observed a clear predominance of lycopsamine- and heliotrine-type PAs, highlighting the occurrence of lycopsamine, intermedine, echimidine and heliotrine. Additionally, a significant presence of europine was mainly detected in retail honey samples harvested in Greece, being the first time that this PA was reported in Mediterranean honeys. This profile of PAs/PANOs observed was in good agreement with the data previously published by the EFSA (EFSA-European Food Safety Authority, 2011) and was well co-related with the specific Mediterranean flora, as the source of lycopsamine-type PAs could be *Echium* spp. and the presence of heliotrine-type PAs can be associated to *Heliotropium* sp. or another *Boraginaceae*. On the other hand, honey samples originated in Belgium were less contaminated (67% of the samples analyzed) and exhibited a different contamination profile than the foreign honeys, as the predominant PAs found in the samples belonged to the senecionine-type (mainly, retrorsine, senecionine, seneciphylline and senecivernine). This contamination pattern is consistent with the ubiquitous Belgian flora, where there are plenty of Senecio species, such as *Senecio vulgaris*. Other authors have also evaluated honeys from a specific geographical areas, such as Spain, Australia and New Zealand, North America, Ireland, Switzerland and China (Orantes-Bermejo, Serra Bonvehí, Gómez-Pajuelo, Megías, & Torres, 2013; Kast et al., 2014; Griffin, Mitrovic, Danaher, & Furey, 2015; Mudge, Jones, & Brown, 2015; Griffin, O'Mahony, Danaher, & Furey, 2015; He et al., 2020). In general, echimidine and lycopsamine were the main PAs found in all these samples. Nonetheless, monocrotaline (from *Crotalaria* spp), a PAs never detected in honey in other regions, was also predominant in contaminated honeys from China (He et al., 2020). Apart from honey, other beehive products used as food supplements (pollen, propolis and royal jelly) and honey-based products (snacks, candies and baby food) have been analyzed to evaluate their degree of contamination with these alkaloids (Kempf, Wittig, Schönfeld, et al., 2011; Mulder et al., 2015, 2018; Picron et al., 2020). Looking at the results described in these works, the 66–91% of the honey-based food supplements analyzed on them were contaminated with PAs/PANOs. The pollen products were clearly the most contaminated in comparison to the levels found in propolis and royal jelly (Kempf, Wittig, Schönfeld, et al., 2011; Mulder et al., 2015, 2018; Picron et al., 2020). Accordingly, concentration levels found for pollen had average values of 555–576 μg/kg, while for the other food supplements (propolis and royal jelly) the values were within 0.6–15.5 μg/kg. Indeed, some authors reported PAs values higher than 1000 μg/kg in some pollen samples. For instance, Mulder et al. (2018) found a 9% of the food supplements analyzed contaminated with these high values, whereas Picron et al. (2020) found one pollen sample (out of 5) contaminated up to 1672 μg/kg. Lycopsamine-type compounds were the predominant alkaloids found in these samples, with clear predominance of echimidine, lycopsamine, intermedine and their *N*-oxide forms, followed by smaller amounts of senecionine-type PAs. Conversely, heliotrine-type and monocrotaline-type compounds were anecdotal. On the other hand, the contamination of honey-based items such as snacks and candies was scarcer than in honey-based food supplements, and positive samples were only contaminated with PAs at low levels (Kempf, Wittig, Schönfeld, et al., 2011; Picron et al., 2020). In this sense, Kempf, Wittig, Schönfeld, et al. (2011) analyzed 10 candies, 7 power bars and cereals, 5 soft drinks, 3 baby food and 3 jelly babies, among other honey-based foodstuffs. From these items, PAs were only found in 2 candies at concentration levels of 10 and 40 ng/g. More recently, Picron et al. (2020) evaluated 39 honey-based snacks (including breakfast cereals, cereals bars and gingerbreads) and 13 candies. Only one third of the snacks were contaminated, and all the cereals bars were free of PAs. The maximum contamination level found in the snack samples was 0.36 ng/g (particularly, in a breakfast cereal). Lycopsamine-type PAs were predominant in the contaminated breakfast cereal samples, whereas heliotrine-type compounds were more abundant in the positive gingerbread samples. On the other hand, 46% of the candy samples were free of PAs contamination, and the maximum concentration detected was 7.61 ng/g in a candy based on Mediterranean honey, highlighting its content in echimidine (6.47 ng/g) which matches with the contamination profile previously described for Mediterranean honeys.

The contamination of other plant-derived products, such as cereals and salads, has also been evaluated but to a lesser extent. For instance, the occurrence of PAs/PANOs in cereals is more limited as there are more strict farming control practices to avoid the presence of weeds and foreign seed in the cereal crops (Edgar, Molyneux, & Colegate, 2015). Nevertheless, these alkaloids have been detected in wheat, flour and other grain-based products at low levels, suggesting their contribution to a slow chronic toxicity that should be evaluated and addressed (Azadbakht & Talavaki, 2010; Edgar et al., 2011, 2015; Letsyo, Adams, Dzikunoo, & Asante-Donyinah, 2020). Regarding salads, none of the edible

plants used for salads are known to produce PAs. However, the leaves of some PA-producing plants (particularly, *Senecio vulgaris*) have similar appearance to the leaves of other salad-plants, which may lead to confusion. This is the case of rucola, which has a close similarity with ragwort leaves (a PA-producing plant) (Ma et al., 2018; Picron et al., 2018a). In this sense, Picron et al. (2018a) analyzed 17 samples of pre-packaged salads. The 70% of the samples were contaminated with less than 0.1 ng/g of PAs/PANOs, but 3 samples contained levels of 2.59, 5.20 and 10.47 ng/g. The PAs found were exclusively of senecionine-type, mainly retrorsine, retrorsine *N*-oxide and seneciphylline *N*-oxide. Surprisingly, none of the samples contained rucola according to their label (only mixes of escarole, curly endive, radicchio and lamb's lettuce), so the contamination was assumed to be due to co-harvesting or cross-contamination.

5. Analytical determination

The recent growing interest in evaluating the presence of PAs/PANOs in food has led to a significant increase in the development of analytical methodologies to detect and quantify these contaminants in different food matrices. Table 3 summarizes the different analytical strategies carried out within the last 10 years for the extraction and analysis of PAs/PANOs in different food matrices. As it can be observed, liquid chromatography coupled to tandem mass spectrometry detection (HPLC-MS/MS) is the main technique for the analysis of PAs/PANOs in food samples, followed by gas-chromatography coupled to mass spectrometry detection (GC-MS). Only one work describes the analysis of PAs/PANOs with liquid chromatography coupled to diode array detection (HPLC-DAD) (Moreira, Fernandes, Valentão, Pereira, & Andrade, 2020), while another one employs the novel approach of direct analysis in real-time coupled to mass spectrometry detection (DART-MS) without requiring chromatographic separation (Chen et al., 2021). Although the analysis of PAs/PANOs has been reported in the past with other techniques, such as thin-layer chromatography, nuclear magnetic resonance, capillary electrophoresis, immunoaffinity or ultraviolet-spectroscopic methods (Mattocks, 1967; Birecka, Catalfamo, & Eisen, 1981; Roitman, Benson, & Lundin, 1982; Segall & Dallas, 1983; Bober et al., 1989; Roeder, 1990; Logie, Grue, & Liddell, 1994; Roseman, Wu, & Kurth, 1996; Langer, Möstl, Chizzola, & Gutleb, 1996; Lebada et al., 2000; Lee et al., 2001; Molyneux et al., 1982; Yu, Xu, Feng, & Li, 2005; Yu et al., 2005; Azadbakht & Talavaki, 2010), liquid and gas chromatography have recently been the ones chosen for the analysis of food samples. This is mainly due to the guidelines and recommendations set by the official international regulation authorities, which must be followed to develop sensitive analytical methods which enable the accurate identification and quantification of these alkaloids at very low concentration levels in a wide range of food products. In this sense, the EFSA has established GC-MS and HPLC-MS/MS as the most suitable analytical techniques for the determination of these compounds because of their high sensitivity and selectivity, since limits of quantification of 10 μg/kg (total content of PAs/PANOs) and 0.1–5 μg/kg (individual content of PAs/PANOs) should be reached, respectively, according to the recommendations (EFSA-European Food Safety Authority, 2011; EFSA-European Food Safety Authority, 2017). However, as shown in Table 3, it is not always possible to reach these low limits, mainly due to the complexity of the food matrices, resulting in matrix interferences that negatively affect the sensitivity of the analytical methods. On the other hand, the use of HPLC or its improved variant UHPLC (ultra-high performance liquid chromatography) are preferred over GC, as they do not require derivatization of PAs/PANOs, thus sample preparation is easier and quicker. For derivatization, different reagents have been used, such as methyl--*N*-(trimethylsilyl)trifluoroacetamide (MSTFA), phthalic anhydride in pyridine or heptafluorobutyric anhydride (HFBA) (Table 3). Another issue, according to the EFSA, is that PAs and their corresponding PANOs can co-occur as they are not metabolically interconvertible and both are toxic, so the analytical procedures have to ensure the extraction and determination of both types (EFSA-European Food Safety Authority, 2011; Ma et al., 2018). However, GC cannot be applied for the analysis of PANOs, as they are unstable at the temperatures needed for volatilization. Likewise, there is risk by GC of thermal decomposition of PAs and their alteration by the formation of diesters from monoesters (Mandić et al., 2015; Ma et al., 2018). It should be also considered that in GC, the determination of PAs is not performed individually as it is usual in HPLC or UHPLC. In this sense, PAs/PANOs of the sample are reduced with zinc and lithium aluminum hydride ($LiAlH_4$) solution in THF (Table 3) to obtain their necine base backbone, by which means the total PAs content of a sample can be measured chromatographically by comparing the signal intensity obtained with that of an internal standard (usually heliotrine), expressing the results as a single sum parameter (retronecine equivalents) (Kempf et al., 2011a, 2011b; Cramer & Beuerle, 2012; Kowalczyk, Sieradzki, & Kwiatek, 2018). Nonetheless, despite applying HPLC, some authors have also used zinc to reduce PANOs to their corresponding PAs (Table 3). One of the reasons for using this reduction procedure is the scarcity of commercial standards, although their availability has increased in recent years. This reduction strategy can be considered an advantage as only a few standards are needed to perform the total quantification of PAs. However, the drawback of this procedure is that it does not allow to determine the type of PAs present in the samples or the origin of the contamination source. On the other hand, due to the high number of pyrrolizidine alkaloids (more than 600 different structures), it is very challenging to detect each of them individually. Moreover, commercial standards for some of them are not available and, in general, the ones which are available are usually expensive. Nonetheless, some laboratories have developed analytical methods that are able to detect up to 70 different PAs (including PANOs) (Chen et al., 2019). For this reason, as explained in section 3, the EFSA currently recommends monitoring the set of 17–21 PAs/PANOs previously indicated (EFSA-European Food Safety Authority, 2017; Picron et al., 2018a; Picron, 2018b), as these are the ones most frequently encountered in food and feed. Therefore, the validation of any analytical method developed for the determination of PAs/PANOs in food samples should include at least the evaluation of all these compounds (Fig. 4). Nevertheless, one of the major issues in the individual determination of PAs/PANOs by HPLC or UHPLC is the co-occurrence of isomers, which in many cases leads to coelution, being impossible to achieve their baseline chromatographic separation and their identification by mass spectrometry (as they have the same molecular weight). Within the PAs/PANOs listed by the EFSA to be monitored in food, this is the case for intermedine/lycopsamine and senecionine/senecivernine, as well as their *N*-oxide derivatives. The separation of intermedine/lycopsamine and their *N*-oxides can be achieved with acidic chromatographic conditions, whereas the separation of senecionine/senecivernine and their *N*-oxides is suitable under basic conditions (Avula et al., 2015; Chen et al., 2019). For this reason, works describing the complete chromatographic separation of all the PAs/PANOs recommended by the EFSA including the separation of the isomers are scarce (Izcara et al., 2020; Kaltner, Stiglbauer, Rychlik, Gareis, & Gottschalk, 2019). Indeed, many authors instead of analyzing these 8 isomers only include some of them and exclude the other ones (Avula et al., 2015; Chmit et al., 2019; Wang et al., 2019), while other works carry out acid and basic chromatographic methods separately (Chen et al., 2019).

Regarding the detection technique, mass spectrometry is the most suitable because of its high selectivity, specificity and sensitivity, which enables a correct and unequivocal identification of the target analytes through their mass spectra. Conversely, ultraviolet (UV) detection is more limited for the analysis of PAs/PANOs, as these compounds do not have a characteristic UV spectra (exhibiting only a non-specific UV-maximum at 214 nm) (Ma et al., 2018). Regarding the analyzers used in the mass spectrometry systems, the single quadrupole has been the only one used in GC, employing electron ionization (EI) as ionization source and selected ion monitoring (SIM) as detection mode (Table 3). In contrast, a greater variety of analyzers have been used for the analysis of

PAS/PANOs by HPLC or UHPLC (Table 3). The triple quadrupole (TQ) has been the one most extensively used, followed by analyzers based on the combination of quadrupole and linear ion trap, such as QTRAP or Q-Orbitrap (Table 3). Nonetheless, other works also describe the detection with ion-trap (IT) analyzers and, to a lesser extent, with quadrupole-time of flight (QToF), single quadrupole (Q) and quadrupole-hexapole-quadrupole (QHQ) analyzers (Table 3). In general, the electrospray ionization (ESI) in positive ion mode is the ionization source of choice for the analysis of PAs and PANOs by HPLC (Table 3). The ESI is much more suitable than the atmospheric pressure ionization (APCI) for the analysis of polar compounds. Some authors evaluated both ionization sources, but the ionization of PANOs, which are more polar compounds, was significantly lower in the APCI than in the ESI mode (Orantes-Bermejo et al., 2013). Likewise, better ionization signals for these compounds were obtained in positive mode than in negative mode (Dzuman, Zachariasova, Veprikova, Godula, & Hajslova, 2015). Most of the works reviewed for HPLC analysis used multiple reaction monitoring (MRM) as detection mode (Table 3), as it is very common for all types of analytes when MS/MS is performed. Only 4 works employed high resolution mass spectrometry (HRMS, or full scan) as detection mode, using Q-Orbitrap (Dzuman et al., 2015; Martinello et al., 2017; Vaclavik, Krynitsky, & Rader, 2014) and QToF (Wang et al., 2019) analyzers, which are very suitable for this type of detection. On the other hand, only 2 works carried out SIM mode detection, particularly with a Q (Martinello, Cristofoli, Gallina, & Mutinelli, 2014) and a TQ (Kempf, Wittig, Reinhard, et al., 2011).

For the extraction of PAs/PANOs, the most common techniques are solid-liquid extraction (SLE) or liquid-liquid extraction (LLE) usually combined with a purification step by solid-phase extraction (SPE) (Table 3). As previously indicated, the extraction method should simultaneously extract the PAs/PANOs of the sample. Therefore, it is common to use polar organic solvents or acidified aqueous solutions due to the great polarity of PANOs (Crews, Berthiller, & Krska, 2010). In this sense, 0.05 M sulfuric acid solution has been extensively used as a solvent for the extraction of these alkaloids from different food matrices (Table 3). Nonetheless, hydrochloric acid, formic acid, acidified methanol, dichloromethane and chloroform have also been used as extraction solvents. Moreover, in samples with high lipid content, such as those of animal origin (milk, eggs and meat), it is usually necessary to previously separate the fat content with non-polar solvents, like hexane (Table 3). On the other hand, for the analysis of (herbal) tea infusions, the extraction method is based on an infusion process with boiling water (Schulz et al., 2015; Picron et al., 2018a; Mulder et al., 2018; Chen et al., 2019), which in many cases enables to calculate a possible real exposure scenario to these contaminants. Due to the great complexity of food matrices, a purification process of the sample extract is usually required prior to analysis, with SPE being the technique most widely used to date. For this purpose, strong-cation exchange (SCX) sorbents have been extensively used for the purification of PAs/PANOs from food samples, followed by reversed phase sorbents (mainly based on octadecylsilane ligands (C18)) and mixed-mode sorbents (combination of reversed-phase and cation-exchange interactions) (Table 3). Only a few works directly analyzed the samples without performing sample preparation (Avula et al., 2015; Valese et al., 2016). In many cases, authors basified the sample extract to pH 9.0–11.0 or neutralized it to pH 6.0–7.0 prior to purification, especially when reversed-phase sorbents were used (Table 3), to promote interactions between the analytes and the sorbent.

Other technique used over the past decade for the determination of PAs/PANOs in food samples has been the QuEChERS (acronym of "Quick, Easy, Cheap, Effective Rugged and Safe"). This strategy involves the simultaneous extraction and purification of the samples and it is very suitable to achieve the extraction of a large number of compounds (multi-residue extraction) (Anastassiades, Lehotay, Štajnbaher, & Schenck, 2003). Moreover, this procedure has been recently miniaturized and successfully applied to the analysis of PAs/PANOs in oregano samples, leading to a sustainable analytical strategy which meets the green analytical chemistry principles by significantly reducing the solvents and reagents used by ten times in comparison to the original procedure (Izcara et al., 2020). Accordingly, small amounts of sample (0.2 g), organic solvents (1000 μL), clean-up sorbents (175 mg) and partitioning salts (0.65 g) were used, showing good method performance for the 21 PAs/PANOs recommended by the EFSA with recovery values in the range 77–96% (Table 3). Another recent work also described the microextraction of 9 PAs/PANOs from honey samples using dispersive liquid-liquid microextraction approach (DLLME) (Celano, Piccinelli, Campone, Russo, & Rastrelli, 2019). The analytes were extracted using minimal volumes of organic solvents: 500 μL of chloroform as extractant and 500 μL of isopropyl alcohol as disperser, achieving recovery values from 63 to 103% (Table 3). These works are a promising advance in the development of environmentally friendly analytical methods by performing miniaturized procedures or microextraction techniques for the determination of PAs/PANOs in food samples, which is one of the challenges within the next years (Casado, Gañán, Morante-Zarcero, & Sierra, 2020).

6. Effect of food processing and (culinary) preparation on pyrrolizidine alkaloids

As it has been described in the previous sections, most of the works published to monitor the presence of PAs and PANOs have been carried out mainly for honey, dry teas and food supplements (Table 3). Indeed, these products are the ones the EFSA has evaluated more extensively and, consequently, there is more information available about the incidence of PAs in them (EFSA-European Food Safety Authority, 2017). Nevertheless, it is also of high interest to evaluate how the content of PAs and PANOs may be affected by transforming contaminated ingredients into processed products. These studies are very necessary to perform a more real assessment of the population's exposure to these toxic alkaloids. However, currently, knowledge about the effect that food processing has on the PAs content, including the detection of their transformation products, is very limited. For this purpose, it is important to study the stability of these compounds. Since the beginning of the 20th century, some cases of PAs poisoning have been reported due to the intake of bread prepared with contaminated grains (Kakar et al., 2010; Molyneux, Gardner, Colegate, & Edgar, 2011; Willmot & Robertson, 1920). This suggests that PAs and PANOs may resist the baking procedure, showing some resistance to heat. Likewise, a study carried out in the 70s concluded that the content of PAs in contaminated meat from animals poisoned by a dehydroPA-containing species of *Trichodesma* was not destroyed by cooking, after feeding some puppies with it (Shevchenko & Fakhrutdinova, 1971). Conversely, other authors have reported that the application of heat can influence the tertiary PAs/PANOs ratio and the total content of PAs in different samples (Hösch, Wiedenfeld, Dingermann, & Röder, 1996; Mattocks, 1986). Moreover, as mentioned above, PANOs are unstable at the temperatures used for their volatilization in GC and thermal decomposition for some diesterified PAs has also been described (Ma et al., 2018; Mandić et al., 2015; Mroczek, Ndjoko-Ioset, Głowniak, Miętkiewicz-Capała, & Hostettmann, 2006; Qi, Wu, Cheng, & Qu, 2009). In this sense, if the necine bases of the PA and PANO structures become degraded at high temperatures during food processing, the potential toxicity and hazard to human health from the intake of these compounds would be greatly reduced. With this premise, Rosemann (2007), chap. 4 evaluated the stability of retrorsine (1,2-unsaturated PA) during food processing. For this purpose, maize flour contaminated with a known concentration of retrorsine was used to prepare maize porridge, heating it in a boiling water bath for 3 h. Both the raw material and the final product were analyzed for comparison purposes. The results revealed a reduction in the content of retrorsine in the maize porridge. However, these results were not conclusive, as only a slight decrease was observed, so it was mainly ascribed to the extraction inefficiency due to the formation of emulsions,

rather than to the instability of retrorsine during the cooking process. Moreover, the same procedure was carried out with raw and boiled herbal tea samples. No differences were found among both samples, so it was concluded that the retrorsine concentration was not affected by high temperatures during normal cooking procedures. Conversely, Boppré, Colegate, Edgar, and Fischer (2008) proved that drying pollen contaminated with PAs with heat significantly reduced the amount of PANOs, which were degraded to their corresponding free base primary PAs. Thereby, a degradation process took place, but the total content of PAs remained the same. In contrast, Kaltner, Rychlik, Gareis, and Gottschalk (2018) observed that during the long-term storage of honey contaminated with PAs at 20 °C, the levels of PANOs decreased with the time, without showing interconversion into their corresponding PAs. Based on the results of these last two works, it seems that thermal conditions contribute to the transformation of PANOs into their corresponding PAs.

On the other hand, the culinary preparation of some products, such as teas and herbal infusions, should also be considered in the final content of PAs in these products. However, there are only a few studies that had evaluated this effect. In this sense, Picron et al. (2018a) performed a transfer study using different dry teas which were brewed for 6 min in boiling water. It was observed that the transfer rate of PAs and PANOs from the raw material to the infusion was only 16–28% (except monocrotaline, with 45%) during the brewing process. Therefore, this work highlights the importance of evaluating how the PAs content may vary from the raw materials to the final product. However, there are many parameters that may have influence on the transfer rate during the brewing process of teas and infusions, like the time, the temperature or even the particle size of the leaves. For instance, Chen et al. (2019) evaluated how the extraction efficiency of PAs and PANOs may be influenced by the particle size of the tea leaves. For this purpose, they prepared infusions from intact leaves and comminuted leaves. The results showed that the PAs levels extracted from the comminuted leaves were 1.1–4.1 times higher than from the intact leaves. Thus, this should be considered when developing routine analytical methods to monitor the presence of these contaminants, as the use of comminuted materials may overestimate the concentration of PAs and, consequently, also their exposure and risk. Nevertheless, due to the shortage of works that address this topic, it is not possible to determine a transfer rate with reliability. For this reason, the EFSA currently estimates the concentration of PAs and PANOs in teas and infusions by applying a dilution factor to the data obtained in the dry raw materials considering a 100% transfer rate, although depending on the transfer process this may lead to an overestimation of the intake and exposure of the population to these contaminants. Therefore, more works evaluating the transfer rate are needed.

Besides the effect of heating, some authors have also evaluated other technological treatments like fermentation. In this sense, Kempf, Wittig, Schönfeld, et al. (2011) analyzed candies and mead containing honey as raw materials. They observed that despite being subjected to heat and/or fermentation, these products, still showed PAs values quite above the average of retail honeys, suggesting that the contamination of a raw material (in this case honey) can be carried on to the final product where significant amounts of PAs can still be present. This downstream contamination was also confirmed by Picron et al. (2020) in food items containing honey, such as candies and snacks (including breakfast cereals, cereal bars and gingerbreads). Nevertheless, only one third of these products were contaminated at low levels. In fact, all the cereal bars were free of PAs/PANOs. Cao et al. (2013) also evaluated how the mead production process affected the content of PAs/PANOs. For this purpose, they prepared mead using contaminated honey as raw material with a known amount of PAs/PANOs. The levels of PAs found in the mead samples prepared were about 30% and 70% of the levels in the raw honey. However, it was not clear if this apparent reduction was due to the fermentation process or simply a dilution effect of the whole process.

From all these works it can be concluded that the impact of food processing and of culinary preparation on the content of PAs and PANOs is not clear. Data available so far seem to indicate that this effect is limited, so more studies within this research line are necessary. For instance, it would be interesting to evaluate the impact of different cooking techniques (e.g. baking, frying, boiling, microwaves, etc.) on the PAs content. These studies are essential to know more safely at what concentration these substances can be ingested through the food products that we actually consume, so that the real intake and exposure of these compounds by the population can be established in a more reliable way. Thus, this is a challenge of great importance for the coming years in the food safety field.

7. Conclusions and future perspectives

In the last decade, many efforts have been made to address the food safety issue of pyrrolizidine alkaloids. In this sense, due to the food alerts notified in recent years, maximum concentration levels have been regulated for food products likely to be contaminated with these alkaloids, such as teas, herbal teas, honey, pollen, aromatic herbs, some spices and food supplements. In this sense, honey has been the food item most extensively analyzed within the last years. However, it is also necessary to determine the occurrence of these compounds in other food matrices less studied to date or which have not been considered by the international guidelines, such as products subjected to different technological procedures (e.g. bread, bakery products, snacks, etc.). In addition, data about thermal stability of PAs are limited and not conclusive, so more works evaluating the transformation or degradation of PAs from raw materials into processed products would be desirable. Thus, further investigation is required regarding food processing and culinary preparation to achieve a reliable assessment of the real intake of these alkaloids by the population and improve the risk management of these contaminants. Likewise, more toxicity studies of the different PAs congeners are needed to evaluate a possible chemical structure – toxicity relationship. It is also of high importance to develop sensitive, selective and environmentally friendly analytical methods that can be properly validated to achieve a correctly identification and quantification of these compounds. Currently HPLC-MS/MS is the main technique for the analysis of PAs/PANOs in food samples, as it enables to perform an individualized study to determine the type of PAs present in the samples analyzed, as well as the contamination source. However, this is a challenging task due to the high number of PAs structures and the scarcity and price of the standard solutions for their quantification. Indeed, the EFSA currently proposes to monitor a set of 17–21 PAs/PANOs, as to date they are the ones most frequently encountered in food and feed. However, this is only a small percentage of the total PA structures. Thus, this might be a blind spot, as other PAs or possible metabolites may also be present in food but are not been considered so far. On the other hand, another important issue in the individual determination of PAs/PANOs by HPLC is the coelution of isomers. Many efforts are being made to address this challenge in order to develop advanced analytical methodologies that contribute to quantify and detect these contaminants in a sensitive way and ensure food safety.

Author contributions

N. Casado carried out the conceptualization, collected the data and drafted the manuscript. S. Morante-Zarcero and I. Sierra supervised and edited the manuscript. Project administration and funding acquisition was performed by I. Sierra.

Declaration of competing interest

Authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tifs.2022.01.007.

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