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Review

Insight into the Impact of Food Processing and Culinary Preparations on the Stability and Content of Plant Alkaloids Considered as Natural Food Contaminants

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Abstract: Pyrrolizidine alkaloids, tropane alkaloids and opium alkaloids are natural plant toxins that have recently gained special interest in food safety due to their concerning occurrence in many foods and feeds. Although a legislation for these alkaloids has recently been established, the concentration levels of these toxins in food exceed in many cases the maximum limit established by the competent authorities. Moreover, these regulations only establish maximum limits of these compounds for certain raw materials, but processed products are generally not considered. However, it is important to correctly assess the potential health risk of these alkaloids through the diet. Accordingly, this review aims to provide insight into these alkaloids and give an overview on how food processing and culinary preparation can influence their content and stability. For this purpose, the most relevant works that address the effect of heat treatment, fermentation, infusion preparation (transfer rate) and other treatments (milling, washing and soaking) on these natural toxins are reviewed. To date, this research field has been scarcely studied and many of the results published are contradictory, so it is not always possible to establish conclusive findings. In many cases, this is due to a lack of experimental design and exhaustive control of the different variables that may affect these treatments and preparations. Likewise, considering the transformation of these alkaloids into toxic degradation products it is also of high interest. Therefore, further studies are needed to delve deeper into the stability of these toxins and to understand how their content may be affected by the transformation of contaminated raw materials into processed products, so that the risk exposure of the population to these alkaloids through diet can be determined more precisely. Hence, this topic constitutes a research line of great interest for future works with many challenges to be resolved.

Keywords: plant toxins; pyrrolizidine alkaloids; tropane alkaloids; opium alkaloids; food processing; food safety; culinary process; stability

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1. Introduction

Food safety is one of the main concerns of the food industry, in which there are still many issues to be resolved and challenges to face in order to detect and prevent possible food risks. In fact, food safety is part of one of the Sustainable Development Goals (SDGs) included in the 2030 Agenda, specifically SDG 2, which seeks to end hunger, achieve food safety, and improve nutrition, as well as promoting sustainable agriculture [1]. However, this SDG is also complemented with others that may be indirectly related to food issues, such as SDG 3 (ensure healthy lives and promote well-being for all at all ages) and SDG 12 (responsible consumption and production) [1]. In this sense, to ensure food safety, it is necessary to carry out an exhaustive control of the entire food supply chain, which covers all the steps of the food production and processing process, from the moment the food products are obtained until they reach the final consumer, because all the steps are decisive in the quality of the final product. Accordingly, the European Farm to Fork strategy seeks

to make food systems fair, healthy and environmentally friendly based on the premise that the combination of new technologies and scientific discoveries with increasing public awareness and demand for sustainable food can benefit all stakeholders [2]. For this reason, in recent decades, food safety has evolved towards the demand for greater food control through the development of new production systems, technologies and eating habits. This has enabled the detection of new risks, different from classical contamination by microorganisms, which are caused by the occurrence of contaminating substances whose presence in food has gone unnoticed to date. Moreover, the fact that the health of consumers may be affected by food intake has led to a revision of food legislation, highlighting the need to redirect regulations towards a policy more focused on surveillance [3].

Currently, there are five types of organic contaminants legislated in Europe that are monitored in different food groups to ensure safe food products from "farm to table". These five types include: (1) contaminants from food processing, industrial activity, environmental pollution, mycotoxins and other natural toxins, (2) pesticide residues, (3) veterinary drug residues, (4) residues of compounds that act as growth promoters and (5) contaminants from packaging [4,5]. Within the first group, some natural plant toxins have been considered in recent years as priority pollutants. Among them, pyrrolizidine alkaloids (PAs), tropane alkaloids (TAs) and opium alkaloids (OAs) have recently gained special interest due to their occurrence in many foods and feeds [6–8]. As a result, many food alerts have notified the presence of these alkaloids at concentration levels higher than the maximum levels established by the competent authorities in food, which could lead to the appearance of chronic diseases or acute intoxications [6–9]. Accordingly, the foods more likely to be contaminated with these alkaloids are those of plant origin, such as cereals, seeds, herbs, spices, teas, herbal teas, honey, food supplements and their derived products. For instance, high levels of PAs, up to 133,870 μg/kg, have been detected in oregano samples, while in the case of TAs, concentrations in the range 1200–1500 μg/kg and 360–460 μg/kg of atropine and scopolamine, respectively, have been noted in sorghum flours. Likewise, concerning levels of the OA morphine, ranging between 2.4 and 5.2 mg/kg, have been reported in frozen bread with poppy seeds [9].

For this reason, a legislation for these alkaloids has recently been established, which allows guaranteeing the monitoring and control of these contaminants in several foods [10–12]. Nonetheless, these regulations only establish maximum limits of these compounds for certain products regarded as raw materials, but do not consider the amount of these alkaloids that may be present in the final product after being subjected to food processing or culinary preparation. Accordingly, there are many products that after acquiring them from the supermarket they are afterwards subjected to some type of processing or culinary preparation (e.g., boiling, frying, baking, etc.) before their intake, such as the preparation of pasta, tea, bread, etc. For instance, in the case of tea the current legislation of these alkaloids is referred to their content in dry tea leaves but does not consider whether the subsequent preparation of the infusion may affect the stability of these toxins. Thus, different processing and preparation procedures to which foods are subjected may change the content of these alkaloids, producing their degradation or even their transformation into other compounds. These are important aspects to be considered as they directly affect the safety of foods. Therefore, it is interesting to evaluate the stability of these toxins to see how their content may be affected by transforming contaminated raw materials into processed products, so that the risk exposure of the population to these alkaloids through diet can be determined more precisely [6–8,13].

To date, many works have reviewed the occurrence and analysis of these alkaloids in foods [6–8]. However, despite the importance of assessing the effect of food processing on these compounds, only a few recent reviews have addressed this issue [6,13]. Nonetheless, only one focused on PAs [6], while the other only deals with the determination of the degradation or transformation products derived from heat treatment or storage [13]. Accordingly, the aim of this review is to give an overview of these natural plant toxins (PAs, TAs and OAs) and carry out a revision of the most relevant works which have evaluated

how food processing and culinary preparation may have an influence on their content and stability. In this sense, the current state of knowledge regarding the effect of thermal treatment, fermentation, infusion preparation (transfer rate) and other treatments (grinding, washing and soaking) is reviewed. Likewise, challenges and expected future outlooks are also included.

2. Natural Toxic Plant Alkaloids with Relevant Occurrence in Food

Natural toxins produced by plants are an important group of organic contaminants that can frequently appear in foods unintentionally. Although they are natural products, they are considered contaminants due to their high toxicity and the relevant negative impact that their intake may have on human and animal health [14–17]. Among these toxins, it is worth highlighting the alkaloids, which are a very diverse group of nitrogenous compounds synthesized by many plants as secondary metabolites, mainly from amino acids [18]. Within the known families of alkaloids, PAs, TAs and OAs have received special interest in recent years due to their concerning occurrence in different food products, which can cause mild disorders (acute intoxications) to serious situations (appearance of chronic diseases or even death) [6–8]. In general, these alkaloids are produced by plants that grow in fields as weeds and contaminate food crops, leading to their appearance in the production of plant-derived food products. Nonetheless, their occurrence has also been detected in animal-derived food products, as these contaminants can be transferred when they are consumed by animals, since many of these plants may contaminate feed or be present in forage fields where animals are fed [6–8]. Figure 1 shows the evolution in the number of food alerts related to the presence of high levels of PAs, TAs and OAs in the last 10 years (from 2012 to 2022), as well as the distribution of these alerts according to the type of product contaminated.

2.1. Origin, Toxicity and Occurrence of Pyrrolizidine Alkaloids (PAs)

Pyrrolizidine alkaloids (PAs) and their *N*-oxides (PANOS) are toxic secondary metabolites of plants synthetized as a defense against insects and herbivores. It has been estimated that these alkaloids may occur in more than 6000 plant species worldwide, representing 3–5% of all flowering plants. Accordingly, the main PA-producing plants are the ones belonging to five main families: Asteraceae (tribes Senecioneae and Eupatorieae), Fabaceae (genus *Crotalaria*), Boraginaceae, Orchidaceae and Apocynaceae [19]. The content of PAs in these plants is highly variable and depends on many factors, such as: the species, the plant organ, growing conditions, harvesting, storage, etc.

The intake of PAs/PANOs involves a potential health risk because they can possess either acute or chronic toxic effects [20]. In accordance, the structure of PAs is an important prerequisite for their toxicity. PAs have a common backbone chemical structure, so-called necine, which includes two pyrrole rings fused to a nitrogen heteroatom at position 4 (Figure 2a). This necine part can be esterified with miscellaneous acids, leading to an acidic part called necic acid (Figure 2a). Depending on the type of necic acid bound to the necine base, many different structures can arise in nature. Accordingly, more than 600 different structures for PAs and PANOs are currently known [19]. Moreover, the necine base can either have one unsaturation in the 1,2-position or be saturated, leading to two main groups of PAs: 1,2-unsaturated PAs and saturated PAs, respectively (Figure 2a). In addition, depending on the esterification of one or both hydroxyl groups, 1,2-unsaturated PAs can present monoester, open chain diester and macrocyclic type forms.

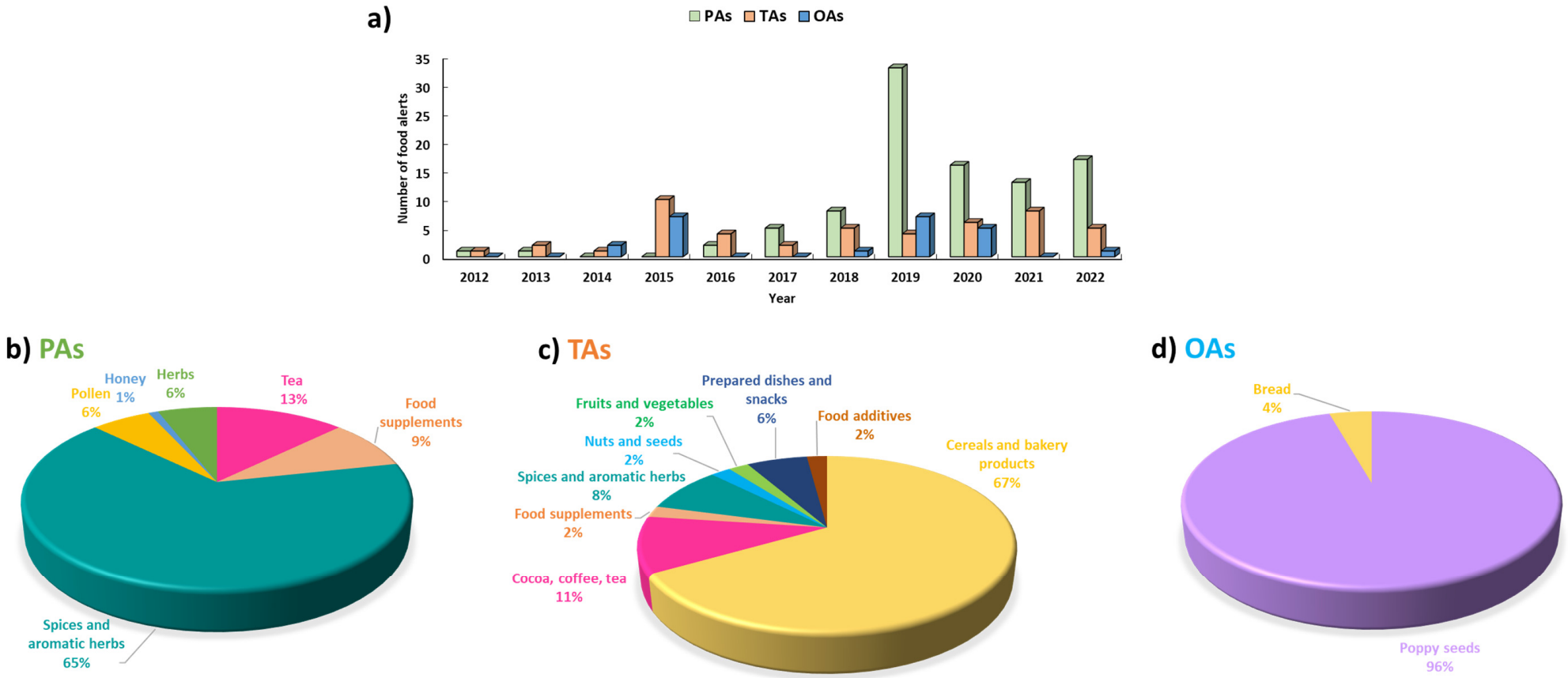

Figure 1. (**a**) Evolution in the number of food alerts that have noted high levels of pyrrolizidine alkaloids (PAs), tropane alkaloids (TAs) and opium alkaloids (OAs) from 2012 to 2022; Distribution of food alerts related to (**b**) PAs, (**c**) TAs and (**d**) OAs based on the food type contaminated (data obtained from Rapid Alert System Feed and Food (RASFF) portal, 2022 [9]).

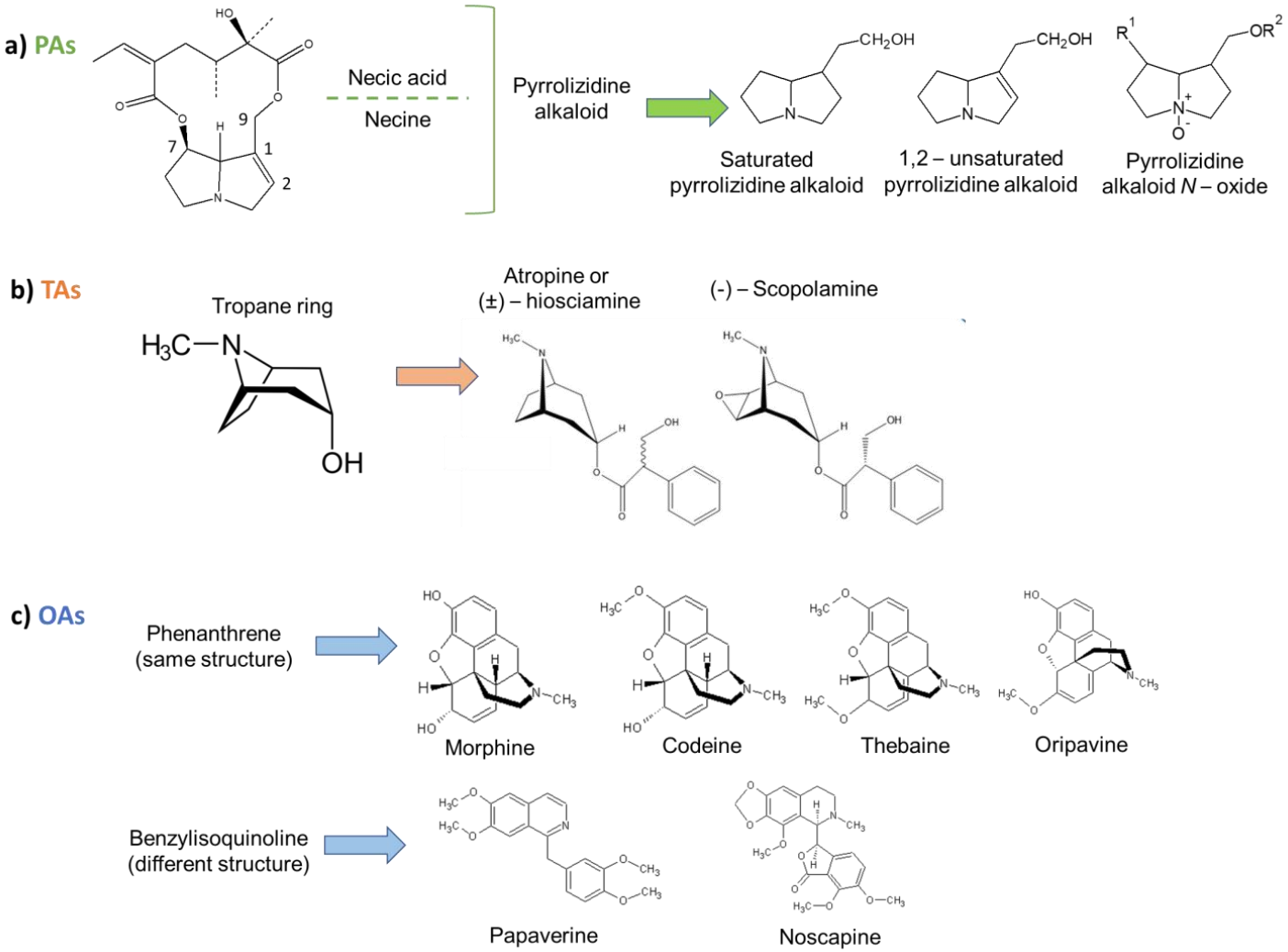

Figure 2. (**a**) Common chemical structure of pyrrolizidine alkaloids and different forms for pyrrolizidine alkaloids (R1 and R2 correspond to different necic acids); (**b**) General common chemical structure of tropane alkaloids (TAs), and chemical structure of the main TAs: atropine and scopolamine; (**c**) Chemical structures of the two families of opium alkaloids (phenanthrenes and benzylisoquinolines).

The unsaturation in the necine base increases the toxicity of these alkaloids. Hence, saturated PAs are not considered toxic compounds, as they do not undergo metabolic activation in the body to exert toxicity. In contrast, 1,2-unsaturated PAs are metabolically activated into highly reactive pyrrole intermediates that act as alkylating agents of proteins and nucleic acids forming cellular adducts, which are responsible of the hepatotoxic, hepatocarcinogenic and genotoxic effects associated with these alkaloids [6,21–23]. Acute PA intoxication can produce abdominal pain, nausea, vomiting, ascites, fever, diarrhea, oedema and jaundice [18]. On the other hand, long-term exposure to PAs is mainly associated with hepatic veno-occlusive disease (HVOD), which involves obstruction of the small veins in the liver with the sudden onset of hepatomegaly and ascites, leading to liver cirrhosis and even liver failure [24]. Moreover, it can also affect the lungs (pulmonary hypertension) or the cardiovascular system (cardiac right ventricular hypertrophy) [21]. Furthermore, high doses of unsaturated PAs can produce fetotoxic and teratogenic effects [18]. In addition, the International Agency for Research on Cancer (IARC) has classified PAs as "possibly carcinogenic to humans" (category 2 B), particularly lasiocarpine, riddelliine and monocrotaline, because of their capacity to bind to DNA and cellular proteins [6,18,21]. Therefore, the frequent intake of these alkaloids entails a potential health risk for consumers. Hence, the analytical control of PAs in food and feed it is of the utmost importance within the food safety field.

PAs can enter the food chain from different vegetables and botanical sources. PA-containing plants can be directly consumed by humans (e.g., borage) and animals (e.g., forage). Nevertheless, the most common sources of PA consumption are usually plant-derived products and feed contaminated with non-edible PA-containing plants, which extensively grow as weeds in crop fields [6]. Therefore, PAs can be introduced into the food chain at early stages of the production process due to harvesting of PA-containing plants. After harvesting, it is very difficult to separate the PA-containing plant parts which produce the contamination of feed and food. Thus, to prevent PA contamination, good and very strict agricultural practices are needed to prevent harvesting the PA-containing plants or even the appearance of foreign plants in the crop [20].

This accidental PA contamination pathway was first widely assumed in an exclusive way. However, recently, different works have revealed that besides cross-contamination during harvesting processes, there are other possible contamination pathways, such as natural horizontal transfer through soil, food fraud and adulteration [6,25–30]. As a result, high levels of PAs have been detected in many food products: aromatic herbs and spices (65% of the food alerts notified), teas and herbal infusions (13% of the food alerts notified), plant-based food supplements (9% of the food alerts notified), herbs (6% of the food alerts notified), pollen (6% of the food alerts notified) and honey (1% of the food alerts notified) (Figure 1b) [6,9]. Likewise, they have also been detected in other commodities, such as flour, cereals, cereal products, salads, milk, dairy products, eggs, meat and meat products, but with lower prominence [6].

As a result of the broad occurrence of PAs in a wide variety of food products and their potential health risks described above, the European Commission published in December 2020 a regulation amending Regulation (EC) No. 1881/2006 to monitor the occurrence of PAs in some food products [10]. This regulation (Commission Regulation (EU) 2020/2040) includes maximum concentration levels of PAs between 1.0 and 1000 μg/kg for several products, such as: teas, herbal infusions, herbal food supplements, pollen, pollen-based food supplements, pollen products, dried herbs and cumin seeds [10]. However, this regulation does not include any product subjected to food processing. Similarly, the preparation of teas and herbal teas (transfer rate) is not considered either, since maximum limits have only been set for dried products and not for infusions (except in the case of teas and infusions for infants and young children) [10]. Therefore, new data must continue to be collected in order to include other foods in the legislation, so that dietary exposure to these contaminants can be more precisely controlled.

2.2. Origin, Toxicity and Occurrence of Tropane Alkaloids (TAs)

Tropane alkaloids (TAs) are toxic secondary metabolites naturally synthesized by different plant families, such as Solanaceae, Brassicaceae, Convolvulaceae and Erythroxylaceae. There are over 200 different compounds identified, all of them characterized by having a common chemical structure known as the tropane skeleton, which involves a two-ring structure with pyrrolidine and piperidine rings sharing a single nitrogen atom and two carbon atoms (Figure 2b) [7,31]. The variety of compounds generally arise by the esterification of tropine with different acids, such as propanoic acid, isovaleric acid, acetic acid, isobutyric acid, (+)-α-hydroxy-β-phenylpropionic acid, tropic acid, among others [31]. The most common TAs that can contaminate food are (−)-hyoscyamine and (−)-scopolamine (also known as hyoscine) (Figure 2b). The racemic mixture of (±)-hyoscyamine is called atropine (Figure 2b), but of the two enantiomers only the (−)-hyoscyamine shows toxicity. Nonetheless, the (−)-hyoscyamine is unstable and undergoes racemization over time, so both enantiomers can appear (mainly in the oldest plant organs) in variable proportions, although they always favor the (−)-enantiomer [32].

The presence of these compounds in food can have serious consequences on the health of consumers, due to their anticholinergic effects. TAs can produce mild or severe symptoms depending on the concentration ingested. Nonetheless, symptoms are generally acute since chronic toxicity caused by these alkaloids has not yet been demonstrated [33]. TAs act as muscarinic acetylcholine receptor antagonists; therefore, they produce several symptoms, such as changes in heart rate, excessive salivation, pupil dilation, a reduction in gastrointestinal tone, hallucinations and even death at higher concentrations [34,35]. These symptoms are generally described for atropine and scopolamine; however, knowledge concerning the toxicity of other TAs is still limited. For instance, tropine increases intestinal contractions, but pseudotropine causes the opposite, an inhibition of intestinal contractions, while cuscohygrine suppresses the immune response in animals [36]. Nonetheless, for the rest of the TAs their toxicity is unknown. For this reason, to date they have been less studied than atropine and scopolamine. Moreover, they usually appear at lower concentrations. However, depending on the species and family, the compounds vary. In this sense, the Solanaceae family is the most problematic as it presents high concentrations of atropine and scopolamine. The Solanaceae family has 90 genera and over 4000 species [35]. *Datura*, *Brugmansia*, *Atropa*, *Hyoscyamus* and *Mandragora* are some of the most concerning genera in this family. Specifically, *Datura stramonium* is one of the species that has generated most of the reported poisoning cases [7,37]. On the other hand, the Convolvulaceae family includes the *Convolvulus* species (commonly known as bindweed), which are rich in TAs such as convolvine, convolidine and other low molecular weight TAs such as tropine, pseudotropine and tropinone [31,36]. Although this family has been less studied than others, it should also be carefully studied as the plants belonging to this family often invade European fields. Therefore, their presence may pose a problem of contamination with TAs other than atropine and scopolamine.

The most common route of TA contamination is related to crops that are harvested at the same time as these toxic plants, as they present similar maturation cycles to some food crops [33]. Moreover, the use of machinery during harvesting, the reluctance to use phytosanitary products and the trend towards organic products increase the contamination of foodstuffs with this type of natural toxin. Likewise, as in the case of PAs, other ways of contamination have recently been proposed, such as the natural horizontal transfer of TAs through the soil. In this sense, rainwater can contribute to this hypothetical horizontal transfer because of the high water solubility of TAs [38].

In 2013, an acute reference dose (ARfD) of 0.016 μg/kg body weight expressed as the sum of (−)-hioscyamine and (−)-scopolamine assuming equivalent potency was set by the European Food Safety Authority (EFSA). Likewise, the EFSA also recommended the collection of data on the presence of TAs in food, on atropine enantiomers, on TA toxicity, and on methodologies covering the analysis of TAs [31]. In 2015, the European Union (EU) recommended the monitoring of TAs in different plant-based foods, such as

cereals and derived products, gluten-free products, tea and infusions, food supplements, legumes and derived products [39]. In addition, it was also indicated that at least atropine and scopolamine should be controlled and, if possible, the enantiomers of hyoscyamine. However, as for analytical reasons it is not always possible to differentiate between the enantiomers of hyoscyamine, maximum levels for scopolamine and atropine have been established, considering that the synthesis of TAs in plants leads to (−)-hyoscyamine and (−)-scopolamine. Accordingly, the EU has recently set maximum limits (0.2–50 µg/kg) expressed as the sum of scopolamine and atropine for different products, including herbal teas, as well as processed and unprocessed cereals from sorghum, millet, corn and buckwheat [11]. Moreover, a maximum limit was set at 1 µg/kg for scopolamine and 1 µg/kg for atropine in processed cereal-based foods and baby foods for infants and young children [40]. However, it must be considered that this means overestimating the potential toxicity of the food, since it has been confirmed that the (+)-enantiomer can appear in the plant over time [32] and that some processing conditions also favor the racemization [41].

Regarding the foods more likely to be contaminated with TAs, almost 70% of the food alerts reported in the last 10 years have been notified in cereals and bakery products (Figure 1c), being millet and maize, the main cereals contaminated with high levels of these alkaloids. Currently, most of the publications related to the analysis of TAs focus on the development of analytical methodologies for their determination in plant-based foods, such as cereals [42], herbal teas [33,43,44], spices, aromatic herbs [45], vegetables [38,46], processed cereal-based products (e.g., biscuits, breakfast cereals) [47,48], etc. However, only a few of these works have evaluated the impact that food processing or culinary preparations may have on the TA content in contaminated samples. Nonetheless, most of these works only report the increase or reduction observed in the concentration levels of these alkaloids subjected to different procedures, but do not investigate the generation or appearance of transformation products [13]. One of the reasons is the lack of knowledge about the true toxicity of many of these alkaloids. Accordingly, the EFSA currently recommends an exhaustive study of thermal processing, transfer rate into infusion waters and degradation products generated by thermal processing along with toxicity studies [31,35]. These topics represent new research lines in the TA field and, to date, only a few works have addressed them, as will be explained in the following sections.

2.3. Origin, Toxicity and Occurrence of Opium Alkaloids (OAs)

Opium alkaloids (OAs) are secondary metabolites found in the milky sap of opium poppy plant (*Papaver somniferum* L.). Its latex (opium) derived from the capsules contains as many as 80 different Oas. However, from all these OAs, the most common are six of them, which can be divided into two groups according to their chemical structure: the phenanthrenes (morphine, codeine, oripavine and thebaine) and benzylisoquinolines (noscapine and papaverine) (Figure 2c) [49]. The phenanthrenes have an aromatic A ring, partially saturated B- and C-rings and a nitrogen-containing D-ring, spanning carbons 9 and 13 (Figure 2c). A common biosynthetic pathway is found; thebaine is the precursor of oripavine and codeine, which are both precursors of morphine. On the other hand, as the name suggests, the benzylisoquinolines consist of an isoquinoline and a benzyl part. They have one methylated nitrogen atom and oxygen functional groups (Figure 2c).

The presence of these alkaloids in opium means that this traditional plant is widely used medicinally for its pharmacological properties. However, its consumption can result in false-positive drug tests and adverse consequences for health, such as nausea and vomiting, drowsiness, respiratory problems, and dependence, especially for the most vulnerable people and more severe cases of intoxication [8].

In recent years, the seeds of this plant have been increasingly used in some foods, such as bakery products (bread, rolls, cookies, etc.), salads or yoghurt toppings, or even to produce teas and oil. Although poppy seeds do not naturally contain OAs, the problem is that they may be contaminated by the latex from this plant during harvesting or by insect damage [50]. However, due to the lack of control, numerous food alerts have been

reported recently (23 since 2012) (Figure 1) noting high levels of morphine in poppy seeds, up to almost 400 ppm [9]. Therefore, due to the potential health risks described above, the European Commission has recently published Regulation (EU) 2021/2142, which came into effect on 1 July 2022 [12]. This regulation establishes maximum levels of OAs (expressed as morphine equivalents calculated as morphine + 0.2 × codeine) in bakery products (1.5 mg/kg) and in poppy seeds (20 mg/kg) [12]. However, since 2018, the EFSA has claimed for new analytical methods that are effective in quantifying the six OAs in different food samples because, to date, most studies have focused on morphine and codeine, but the other OAs may be more toxic, as requested by the health authorities [49].

As a result, the number of studies related to this family of alkaloids has increased in recent years. These studies have been mainly performed on poppy seeds, in which considerably high concentrations above the established maximum limit have been found [51–54]. For instance, recently the six main OAs have been studied in 11 commercial samples of seeds and it was found that all of them were present in all the samples analyzed, with concentrations in the range from 1.5 to 249.0 mg/kg for morphine, from <0.2 µg/kg to 45.8 mg/kg for codeine, from <2.4 µg/kg to 136.2 mg/kg for thebaine, from <0.2 µg/kg to 27.1 mg/kg for papaverine, from <0.2 µg/kg to 108.7 mg/kg for noscapine, and from <240 µg/kg to 33.4 mg/kg for oripavine [54]. Likewise, some works have determined these alkaloids in bakery products [51,55–57]. For example, Casado-Hidalgo et al. (2022) analyzed four slice breads and five breadsticks. In the case of breadsticks, the concentrations found were very low, while concentrations were higher in sliced bread, showing concentrations up to 8.3 ± 0.5 of morphine and 2.4 ± 0.2 mg/kg of codeine, respectively, exceeding the established EU maximum limits [55]. López et al. (2018) analyzed two bakery products, in particular, two cakes, showing up to 0.6 mg/kg of morphine and <0.1 mg/kg of thebaine, codeine, noscapine and papaverine [51]. On the other hand, Carlin et al. (2020) studied poppy seeds without any pre-treatment, observing considerable high amounts of OAs, and then prepared muffins and breads topped with these poppy seeds. However, in the bakery products prepared they did not detect any OAs [56]. In another work by Casado-Hidalgo et al. (2022), bakery products were studied, specifically seven commercial samples of sponge cakes and biscuits were analyzed and in five of them morphine concentrations were below the established maximum limit, while the other OAs were found to be below the limit of quantification [57].

Considering that the six main OAs have been found in high concentrations in some samples, it can be concluded that it is necessary to continue collecting data on the concentration of all OAs in different foods and not only on morphine and codeine, so that additional OAs can also be legislated, because according to health authorities they can be even more toxic. In addition to developing methodologies for the six main OAs, it is also necessary to develop methodologies to determine possible degradation compounds produced as a result of food processing and culinary preparations, as many of the samples that may contain OAs are subjected to treatments such as thermal, fermentation, washing or grinding.

3. Effect of Food Processing and Culinary Preparations on PAs, TAs and OAs

As indicated above, PAs, TAs and OAs have been detected and regulated in many commodities [6–12]. However, it is also of high interest to evaluate the effect of food processing and culinary preparations on these alkaloids. Accordingly, some of the most notable works regarding thermal treatment, fermentation, transfer rate by infusion and other treatments (i.e., grinding, washing and soaking) are described below. Moreover, Figure 3 gathers and classifies a of number works currently available in the literature addressing this issue in the different target alkaloids. Likewise, Figure 4 summarizes the occurrence and effect of food processing and culinary preparation on PAs, TAs and OAs according to the available literature.

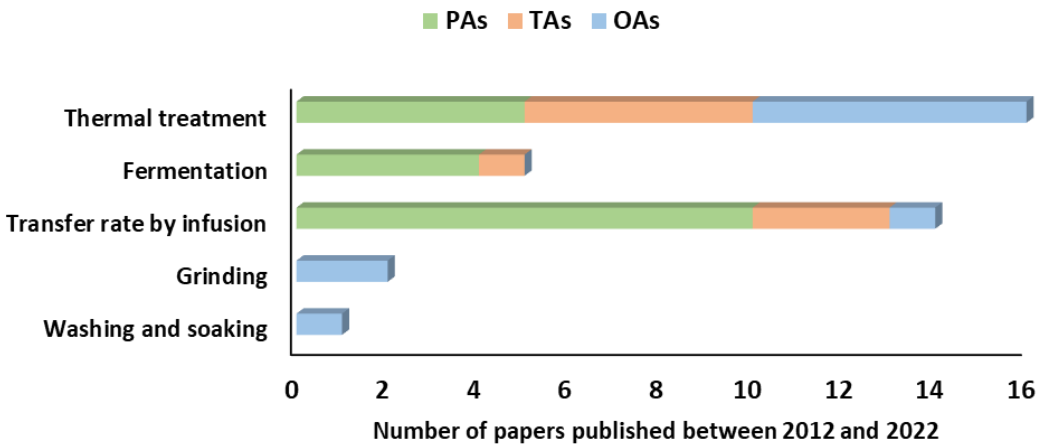

Figure 3. Overview of the number of works currently available in the literature addressing the effect of different food processing and culinary preparations on the content of pyrrolizidine (PAs), tropane (TAs) and opium (OAs) alkaloids. Data obtained from the Scopus, Web of Science and Google search engines from January 2012 to December 2022 (keywords used: transfer rate, food processing, alkaloids degradation, plant toxins, pyrrolizidine alkaloids, tropane alkaloids, opium alkaloids, culinary process, alkaloids stability).

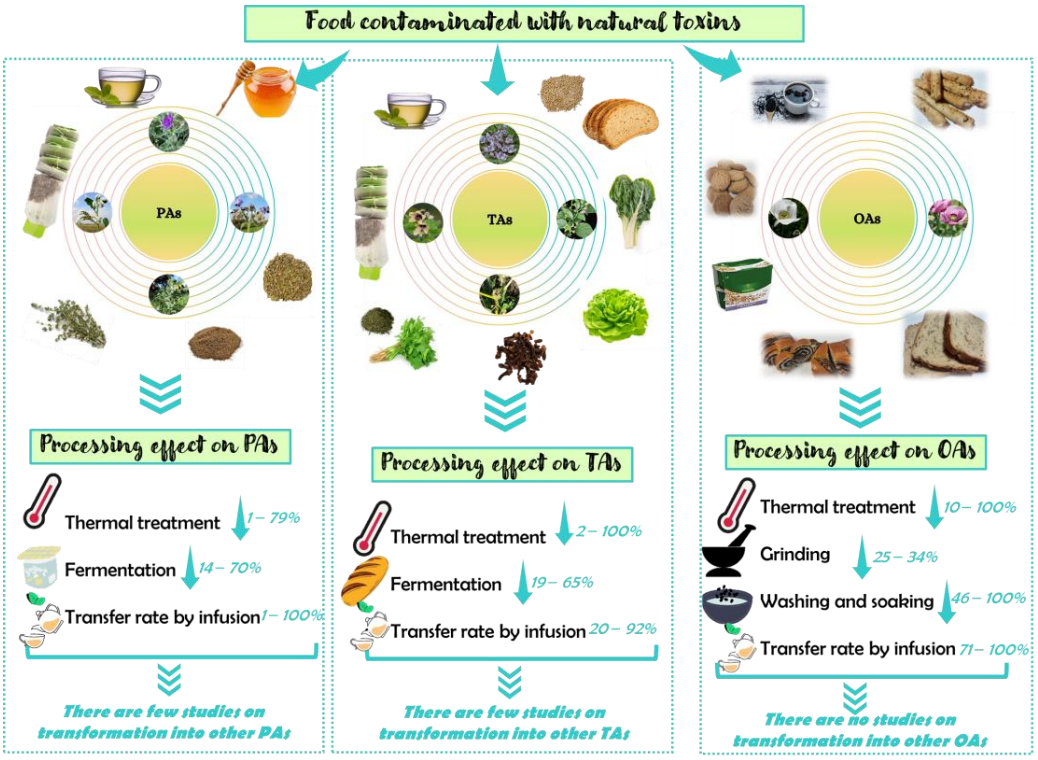

Figure 4. Overview of the occurrence and effect of food processing and culinary preparation on pyrrolizidine (PAs), tropane (TAs) and opium (OAs) alkaloids according to the works included in this revision and currently available in the literature.

3.1. Thermal Treatment

Among the different food processing procedures and culinary preparations investigated, to date, the effect of thermal treatments on the concentration of PAs, TAs and OAs has been most studied (Figure 3). In this sense, the application of heat is a relevant point in food processing and culinary preparations, as it is applied in a wide variety of industrial processes to which many raw materials are subjected. Table 1 collects the works which have addressed the effect of thermal processes on the concentration of these alkaloids. As

observed, several heat treatments have been applied to different samples. For instance, milk processing involves the application of thermal treatments with different degrees of heat to eliminate pathogenic microorganisms (e.g., pasteurization, UHT, sterilization). Likewise, during tea manufacturing a drying step of the plant material is required. Additionally, the manufacture of bread and bakery and pastry products involve baking processes, in which high temperatures are reached. On the other hand, sometimes the intake of a product requires prior culinary preparation that involves heating, such as the preparation of a pasta dish, which requires a boiling procedure. Therefore, it is worth considering the effect of these thermal treatments carried out in food processing and culinary preparations on the stability of these alkaloids, so that the risk exposure of the population to these alkaloids through diet can be determined more precisely.

The thermal stability of PAs is a matter of interest because if the necine base of their backbone structure becomes degraded with high temperatures, their toxicity could be greatly reduced, thus minimizing the risk associated with their oral intake. In this context, it is known that PANOs are unstable at volatilization temperatures, as they cannot be determined by gas chromatography [58,59]. Likewise, thermal decomposition of PAs has also been described at these temperatures [58–61]. However, the heat stability of PAs is not clear, since the available works that have addressed this issue show inconclusive and contradictory results among them. For instance, different PA intoxication cases caused by the intake of bread prepared with contaminated cereals and flours have been reported [62–65]. Similarly, PA intoxication of puppies produced by the intake of contaminated cooked meat was also confirmed [66]. Accordingly, these works suggest the important heat stability of PAs against the high temperatures used in the baking and cooking processes. Likewise, the effect of other heat treatments, such as pasteurization and sterilization (UHT), on the PA content have been evaluated in milk samples [67]. The results obtained reveal that PA concentrations in the starting milk (thermized milk subjected to 68 °C during 13 s) remained the same as the pasteurized and sterilized milk. Thus, it was concluded that heat treatment had no influence on the PA concentration during milk thermal processing [67]. Therefore, a certain stability of PAs to milk processing can be assumed, as other authors have also detected PAs in heat-treated milk [68,69]. Jansons et al. also confirmed the stability of these compounds at 100 °C by performing a stability study with standard solutions of PAs [70]. However, Rosemann (2007) evaluated the thermal stability of retrorsine (a 1,2-unsaturated PA) in contaminated maize flour while preparing maize porridge. The flour was boiled with water for 3 h. After this thermal process, a slight reduction in the content of retrorsine was observed in the maize porridge, suggesting its degradation by heat application [71]. Nonetheless, these results were not conclusive, as it was not clear if the reduction observed in the retrorsine content was due to heat degradation or to an extraction inefficiency caused by the formation of emulsions [71]. On the other hand, Han et al. also reported degradation of both PAs and PANOs during tea manufacturing, specifically during the drying stage [72]. However, the degradation of PANOs was sharper than the degradation of PAs, with degradative percentages in the ranges 58.2–79.2% and 0.8–26.4%, respectively [72]. Nonetheless, they also showed a simultaneous increase in PAs with a reduction in PANOs during the withering step, suggesting PANOs would be transformed to their corresponding PAs [72]. Accordingly, it is important to highlight that although these compounds may not degrade or completely disappear at high temperatures, they do not remain unchanged either. In this context, some works have described that the tertiary PAs/PANOs ratio and the total content of PAs can be modified with the application of heat [73,74]. Likewise, the transformation of monoester PAs to diesters by heat action has also been reported [58,59]. In the work carried out by Boppré et al. it was confirmed that the content of these alkaloids in a contaminated pollen sample decreased when it was dried with heat [75]. However, the degradation was mainly observed in the PANO content (up to 30%). However, a concomitant increase (150–190%) was determined in the PA free bases [75]. This was attributed to the fact that PANOs were degraded by heat into their corresponding free base primary PAs, maintaining the total levels of PAs [75]. In the same line, Han et al. confirmed the

heating process, such as drying, contributed to PANO reduction and their transformation to their related PAs, while PAs were more stable so they were difficult to oxidize to their corresponding PANOs [72]. In contrast, it was observed that storing contaminated honey with PAs at 20 °C long-term did not decrease the content of PANOs [76]. Likewise, no interconversion to their corresponding PAs was observed under these conditions [76]. Kast et al. (2019) also evaluated the stability of PAs at two different temperatures (15 and 30 °C) in bee bread samples [77]. It was observed that the overall PA content remained relatively stable at 15 °C, while at 30 °C it decreased by 33–39%, mainly due to a reduction in the PANO content. Thus, it seems from the data of these works, that heat may produce a certain degree of thermal degradation, but it can also induce the reduction of PANOs into their corresponding free base primary PAs. Nonetheless, since in the work of Kast et al. (2019) the content of tertiary PAs did not increase accordingly in the stored bee bread, authors excluded a simple reduction of PANOs into their corresponding tertiary PAs. Thus, they suggested that PANO reduction might be caused by the digestive enzymes that bees added to their honey during the production of bee bread [77]. Overall, as can be deduced from all these works, there is much controversy about the effect of heat on the content of PAs and PANOs. Many of the results published are uncertain and inconclusive. One of the main reasons is that many of these works have not carried out an exhaustive control of the variables and parameters that can affect this process. Likewise, none of these works include the study of kinetics and thermodynamic parameters related to the thermal degradation of PAs. On the other hand, it is also important to determine if the degradation reported is real and the compounds are being completely eliminated or if they are being transformed in other ones. As has been indicated, some authors observed the interconversion of PANOs to PAs, but it is also important to determine if they can be transformed into other different degradation products. Accordingly, if this is the case, it will be important to study the toxicity of these new compounds formed as a result of the thermal process, since they might exert even higher toxicities. Thus, the regulation of these degradation compounds should be considered and included in the corresponding legislation.

On the other hand, the thermal stability of TAs has also been evaluated in some works (Table 1). In this context, the first study addressing the effect of temperature on samples contaminated with TAs was carried out by Friedman and Levin in 1989 [78]. This research was focused on the analysis of bread prepared with *D. stramonium* seeds. The bread was separated into crumb and bark, and each part was analyzed separately. The results showed an atropine degradation of 25% in the crumb and 18% in the bark, while scopolamine degradation was of 13% in the crumb and 28% in the bark [78]. The results obtained in this study showed a slight degradation of the TAs analyzed, suggesting they may resist the baking procedure. In contrast, Marin-Sáez et al. (2019) evaluated the effect of baking (190 °C) on different TAs in vial trials (standard solutions under simulated bread-making conditions) and bread prepared with buckwheat and millet contaminated with *Datura stramonium* and *Brugmansia arborea*. In the vial trials, the results showed almost complete degradation of TAs (between 94 and 100% degradation). However, in the contaminated bread samples lower degradation of TAs was reported (between 11 and 100% degradation for *D. stramonium* and 30 and 100% for *B. arborea*), suggesting the food matrix may act as a protection against the degradation of TAs [79]. For this reason, it would be suitable to compare different matrices and evaluate how temperature affects these alkaloids in them. Furthermore, it was observed that the most complex TAs (e.g., atropine, scopolamine, homatropine) degraded to low molecular weight TAs, such as tropine and tropinone. Accordingly, these compounds could be used as potential markers of TAs in heat-treated foods. Nonetheless, how the concentration of some compounds, like aposcopolamine or apoatropine, increased during the baking procedure was also observed, possibly due to the degradation of the most complex TAs into these molecules. In this sense, the partial conversion of the main TAs (atropine and scopolamine) to other degradation products, such as aposcopolamine and apoatropine, and from aposcopolamine to apoatropine, compounds even more toxic than the initial atropine and scopolamine TAs,

was also confirmed [79]. Thermal degradation of TAs was also evaluated in breadsticks samples prepared with corn flour contaminated with *D. stramonium* seeds [80]. In this case, it was observed that the baking process produced the degradation of TAs by up to 65%. Baking at 180 °C for 20 min led to the degradation of atropine between 7 and 65%, depending on the preparation conditions used, whereas under these baking conditions the degradation of scopolamine and anisodamine was between 35 and 49% [80]. Overall, the data obtained in these works seem to indicate that baking can contribute to reducing the content of TAs that generally appear in high concentrations, such as atropine and scopolamine. Nonetheless, the wide variation in the results observed among the different works may be due to the inadequate control of critical parameters in the baking process. Thus, more work evaluating the different parameters (e.g., temperature, baking time, type of food, presence of additives, etc.) should be carried out to draw reliable conclusions. On the other hand, the thermal degradation of TAs was also evaluated under boiling conditions in homemade fresh pasta and tea samples contaminated with *Datura stramonium* and *Brugmansia arborea* [81]. The results achieved were also compared with ones obtained using standard solutions in vials subjected to the same boiling conditions. The pasta was cooked under boiling conditions (100 °C for 10 min) and the tea was prepared at 100 °C and left to cool for 5 min. It was observed that the final concentration of TAs decreased in the standard vials as well as in both samples treated with hot water (100 °C), obtaining 24–66% degradation in pasta, but observing a transfer of the compounds of between 20 and 60% to the cooking water, and 2 and 87% in tea samples. In the case of pasta, the transfer of TAs to the water during cooking could be considered a positive aspect to reduce the TA content in food. Nonetheless, it was also observed that the concentration of some analytes (e.g., aposcopolamine, apoatropine, ecgonine and tropine) increased in the tea samples, as previously reported for bread samples [79]. Likewise, the same degradation products were generated as in the case of baking (generally tropine and tropinone) [81]. According to these results, it can be concluded that TAs seem to have a certain sensitivity to heat. Nonetheless, the degradation routes are not entirely clear, so more studies are needed to confirm these theories. Moreover, the evaluation of different culinary processes involving temperature (e.g., microwaves, oil and air fryers, etc.) should be studied. Likewise, different matrices should also be analyzed, because the shape and composition of the food can prevent or contribute to the degradation of TAs, as well as the ingredients contained, because some of them may enhance the degradation or transformation of TAs. Another relevant point which is worth considering about TAs is the chirality issue. This is of great importance as in the case of atropine, in which one of the enantiomers is toxic while the other is not, as previously mentioned. The effect of temperature, pH, and other parameters, such as humidity, exposure time, etc., can contribute to racemization. Accordingly, Marin et al. [41] evaluated the effect of temperature (30, 50 and 80 °C) and pH (3, 5, 7 and 9) on the racemization of atropine in seeds of TA-producing plants (*Datura stramonium* and *Brugmansia* seeds) and contaminated buckwheat. It was observed that at high temperatures (80 °C) and pH 9, the racemization of this TA takes place. Regrettably, to the best of our knowledge, there are no more works that have delved into this topic. Therefore, more studies about how heat treatments and food processing may affect TAs are needed, not only to broaden knowledge about the generation of degradation products and their toxicity, but also to control their possible racemization.

Regarding the effect of thermal treatment on OAs, as previously mentioned, the amount of this type of alkaloid found in foods subjected to baking processes (e.g., breads, biscuits, sponge cakes, etc.) seems to be less than the concentrations of OAs detected in poppy seed samples (which are well above the maximum legislated limits). This decrease in the concentration of OAs observed in bakery products containing poppy seeds compared to the concentrations quantified in untreated seeds, suggest that food processing may produce the degradation of OAs, specifically at the high temperatures used during the baking process. In 2014, the European Commission (EC) published recommendations on processing practices to eliminate or reduce the presence of morphine in poppy seeds, indicating that the

combination of poppy seed pre-treatment (washing) with food preparation (baking) could lead to an overall 80–100% reduction in OAs in the final product [82]. This aspect has been investigated by some authors; however, the few existing works only focus on morphine and codeine, without considering the rest of the OAs, which can be even more toxic according to health authorities [49]. Moreover, depending on the food matrix and heating conditions, uncertain results have been recorded, ranging from the almost total degradation of OAs to almost no degradation [13]. For instance, in the work of Carlin et al. (2020) a comparison was made between the concentrations found in poppy seeds and those found in bakery products (muffins and rolls) prepared with poppy seeds [56]. Lower concentrations were identified after baking, with near to a 100% reduction observed for morphine, papaverine, codeine, and noscapine in the bakery products, especially in muffins cooked in the oven at 180 °C for 15 min. In addition, it was found that when poppy seeds were subjected to high temperatures on the surface of muffins, the concentrations of the OAs were even lower [56]. Likewise, Sproll et al. (2006) evaluated the effect of high temperatures on the codeine and morphine content in a poppy cake sample (180 °C, 20 min) [52], showing a significant reduction in both analytes, in particular, 50–84% for morphine and 50–90% for codeine. They also observed that when the poppy seeds were used as a baking topping (for poppy buns), exposed to the highest temperature (220 °C), morphine and codeine were reduced by 97 and 93%, respectively [52]. In the work of Vera-Baquero et al. (2022), the thermal degradation of five OAs (morphine, codeine, thebaine, papaverine and noscapine) was investigated in breadstick samples made with corn flour and decorated seeds of *Papaver somniferum* L. [80]. Seven different samples were prepared in total. Studies of thermal degradation showed a decrease in OA content under baking conditions (180 °C for 20 min) between 14 and 58% for thebaine, noscapine and papaverine, and up to 100% for codeine and morphine. As reported in previous articles, the results obtained in this work also evidenced that the thermal degradation of morphine and codeine was higher when the poppy seeds were added to the breadsticks as a topping [80]. Thereby, from the data of these works, it can be assumed that besides heating conditions, it is also important to consider the location of the poppy seeds in the product when applying the thermal treatment. Nevertheless, in the work of Shetge et al. (2020) poppy seeds were subjected to both dry and vapor heat and baking treatments to determine the degradation rate of morphine, codeine, and thebaine [83]. This work is the only one in which the degradation kinetics of heat treatment were studied, apparently obtaining first-order degradation kinetics within the temperatures tested (120–200 °C). The results of the thermal degradation indicated that these OAs were relatively stable. At 120 °C, minimal, if any, degradation of the alkaloids was observed after 120 min. On the other hand, at 200 °C, the stability of morphine and codeine was maintained for approximately 32 to 39 min, while that of thebaine was only maintained for 3 min. Post hoc pairwise mean comparisons of the activation energy (Ea) revealed that codeine had a lower value than thebaine or morphine, while thebaine presented the highest Ea [83]. For this reason, Kleinmeier et al. (2020) and Kuntz et al. (2021) pointed out this controversial topic in the literature [84,85]. It has been suggested that this controversy may be caused by the highly heterogeneous content of OAs in poppy seeds, which makes it difficult to approach these studies, also claimed by Shetge et al. (2020), as relative errors higher than 30% were obtained in their work, especially for morphine [83]. In addition, Kuntz et al. showed that the highest recovery value of morphine in cakes decorated with poppy seeds without grinding was 50% at 180 °C for 20 min, whereas the lowest recovery value for cakes with ground poppy seeds was only 16% [85]. Furthermore, Kuntz et al. reported that the stability of OAs could be the result of insufficient heat exposure, since most of the studies performed regarding this issue had not considered the temperature reached inside the product, thus the matrix could protect the OAs from degradation [85]. In this sense, special care should be taken in future experimental designs, and in monitoring the critical parameters of the baking process, such as heat distribution, to avoid uncertain results in the degradation of OAs [85]. Therefore, there is still much to be explored and conclusions to be drawn about the thermal stability

of OAs. Overall, from the works published, it seems that thermal treatment influences the degradation of OAs, as the content of these alkaloids is lower in treated samples than in seeds. Nonetheless, in addition to knowing the thermal stability of these compounds, it is important to know whether degradation compounds can be formed at the temperatures and times to which the food is subjected. If this is the case, it is important to identify them and evaluate their toxicity, because if potentially dangerous compounds are formed, they should be considered so that legislation can be drawn up to avoid the consumption of foods that may cause adverse effects to the consumer.

3.2. Fermentation

The effect of fermentation has been studied to a lesser extent, and only in the case of TAs and PAs, more so in the latter (Figure 3). Nonetheless, the evaluation of this food processing procedure is also of high interest, as there are many raw materials which undergo a fermentation process to obtain different commercial products, such as yogurt, cheese, beverages, etc. Accordingly, it is also important to consider how this process may affect the content of these toxins, since depending on the fermentation conditions and the type of microorganisms used as the starter culture the content of alkaloids may be affected. In this sense, some works have evaluated the effect that fermentation may have on the content of these alkaloids (Table 2).

In the case of PAs, the effect of fermentation has been determined in mead prepared from contaminated honey [86,87]. In one of these works, it was observed that after the fermentation process, significant amounts of PAs were still present in the final product [86]. Conversely, Cao et al. (2013) observed a reduction in PAs (about 30% and 70%) in the mead samples prepared in comparison with the levels determined in raw honey [87]. However, the authors did not confirm if this reduction was due to a degradation caused by the fermentation process or if it was caused by a dilution effect of the whole process [87]. Nonetheless, the effect of fermentation on the content of PAs has also been assessed in yoghurt and cheese, observing in both cases a reduction in the initial PAs levels from the starting milk [67]. In the yoghurt, a reduction of 27% in the total content of PAs was determined after 6 h of fermentation at 42 °C. Regarding cheese, a reduction of 14% was observed during the cheese making process [67]. It was observed that the reduction did not vary from the first step (from milk to whey) to the second (from whey to cheese). Therefore, the reduction was attributed to microbial activity [67]. Likewise, the effect of cheese ripening on the PA content was also evaluated during a 6-week period [67]. The PA content decreased by 38% during the ripening process. Overall, it was concluded that the decrease observed in the PA content of the fermented dairy products was due to the microbial activity, but no mechanism for the microbial action was detailed in this work [67]. Therefore, it could be interesting to investigate the specific effect of bacterial cultures used for commercial dairy processing on PA concentrations. In contrast, Han et al. indicated an increase in the content of PAs and PANOs during the fermentation step of tea manufacturing, observing a marked increase in the content of PANOs [72]. However, the authors did not specify the reason for this phenomenon and mentioned moisture issues during the rolling step of tea manufacturing.

Therefore, due to the sparse and inconclusive data available at present, more studies about the effect of fermentation on the PA content are required because is not possible to determine the effect of fermentation on these alkaloids in a reliable way with the available data in the literature. Nonetheless, such as in the case of thermal treatments, these works have not performed an exhaustive control of the parameters affecting the fermentation process. Likewise, they have not delved in the action of microorganisms. Therefore, these aspects should be considered for future works addressing this treatment.

Table 1. Thermal treatment effects on the contents of pyrrolizidine, tropane and opium alkaloids in food samples according to literature data.

Analytes	Sample	Thermal Treatment Conditions	Effect Observed	Reference
Pyrrolizidine alkaloids				
Re, Jb, JbNO, Jl, Jz, DehydroJc, HydroxyJb, Eruc, Sk, Ot, Fl	Thermized milk	Pasteurization: 76 °C for 15 s Sterilization: 140 °C for 4 s	No significant effects observed	[67]
Re	Maize flour and maize porridge	Heating in a boiling water bath for 3 h	Approximately reduction of 40%	[71]
Re, Sc, Jb, Sp, Im, He, Eu, Sk, ReNO, ScNO, JbNO, SpNO, ImNO, HeNO, EuNO	Green and black teas	Black tea: dried at 110 °C Green tea: fixed at 220–230 °C for de-enzyme and dried at 110 °C	Reduction of 0.8–79.2%	[72]
He, HeNO, Ls, LsNO, Sc, ScNO, Ig, IgNO, Sa	Pollen	Heating at 35, 40 and 60 °C for 72 and 84 h.	Reduction of 30–75%	[75]
Re, Ech, Im, ImNO, Sc, EchNo, ReNO, Ev	Bee bread	30 °C for 6 months	Reduction of 33–39%	[77]
Tropane alkaloids				
At	*Datura stramonium* and *Brugmansia* seeds and buckwheat	Heating at 80 °C and pH 9	Racemization of At	[41]
At, Scp	Bread crumb [a] Bread bark [a]	Baking at 215 °C for 35 min	Bread crumb: Reduction of 13–25% Bread bark: Reduction of 18–28%	[78]
An, Scp, At, Lt, Ap, Hm, Apo, Scpl, Tropi	Bread [b]	Baking at 190 °C for 40 min	Reduction of 11–100%, except Apo and Ap which increased, possibly due to the degradation and transformation of other analytes into these compounds	[79]
At, Scp, An	Breadsticks	Baking at 180 °C for 20 min	Reduction of 7–65%	[80]
An, Scp, At, Lt, Ap, Hm, Apo, Scpl, Tropi	Pasta	Boiling at 100 °C for 10 min	Reduction of 24–66%	[81]
An, Scp, Coc, Benzoylec, At, Lt, Ap, Apo, Hm, Ch, Ecg, Scpl, Tropi, Trpn, Tr	Tea	Water infusion at 100 °C, left to cool for 5 min	Reduction of 2–87%, except Apo, Ap, Ecg and Tropi, which increased, possibly due to the degradation and transformation of other analytes into these compounds	[81]

Table 1. *Cont.*

Analytes	Sample	Thermal Treatment Conditions	Effect Observed	Reference
Opium alkaloids				
Mor, Cod	Cakes Buns	Cakes: Baking at 180 °C for 20 min Buns: Baking at 220 °C for 20 min (toppings)	Cakes: Reduction of 50–90% Buns: Reduction of 93–97%	[52]
Mor, Cod, Th, Pap, Nos	Muffins and Rolls	Baking at 180 °C for 15 min	Reduction of 100%	[56]
Mor, Cod, Th, Pap, Nos	Breadsticks	Baking at 180 °C for 20 min	Reduction of 23–100%	[80]
Mor	Bread	Conditions #1: Baking at 135 °C Conditions #2: Baking at 220 °C	Conditions #1: Reduction of 10–50% Conditions #2: Reduction pf 30%	[82]
Mor, Cod, Th	Muffin	Baking at 120 °C for 120 min	No significant effects observed	[83]
Mor	Cake	Baking at 180 °C for 20 min	Reduction of 55–75%	[85]

[a] Dough fermentation (37 °C, for 45 min, 90% humidity); [b] dough fermentation (37 °C, for 1 h). Anisodamine (An), apoatropine (Apo), aposcopolamine (Ap), atropine (At), benzoylecgonine (Benzoylec), cocaine (Coc), codeine (Cod), cuscohygrine (Ch), dehydrojacoline (DehydroJc), ecgonine (Ecg), echimidine (Ech), echimidine *N*-oxide (EchNO), echivulgarine (Ev), erucifoline (Eruc), europine (Eu), europine *N*-oxide (EuNO), florosenine (Fl), heliotrine (He), heliotrine *N*-oxide (HeNO), homatropine (Hm), hydroxyjacobine (HydroxyJb), integerrimine (Ig), integerrimine *N*-oxide (IgNO), intermedine (Im), intermedine *N*-oxide (ImNO), jacobine (Jb), jacobine *N*-oxide (JbNO), jacoline (Jl), jacozinec (Jz), lasiocarpine (Ls), lasiocarpine *N*-oxide (LsNO), littorine (Lt), morphine (Mor), noscapine (Nos), otosenine (Ot), papaverine (Pap), retrorsine (Re), retrorsine *N*-oxide (ReNO), sarracine (Sa), scopolamine (Scp), scopoline (Scpl), senecionine (Sc), senecionine *N*-oxide (ScNO), seneciphylline (Sp), seneciphylline *N*-oxide (SpNO), senkirkine (Sk), thebaine (Th), tropane (Tr), tropane alkaloids (TAs), tropine (Tropi), and tropinone (Trpn).

Table 2. Fermentation effects on the contents of pyrrolizidine and tropane alkaloids in food samples according to literature data.

Analytes	Sample	Fermentation Conditions	Fermentation Effect	Reference
Pyrrolizidine alkaloids				
Re, Jb, JbNO, Jl, Jz, DehydroJc, HydroxyJb, Eruc, Sk, Ot, Fl	Yoghurt	0.02% *v*/*v* starter culture (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *Bulgaricus*) 42 °C for 6 h until pH 4.4	Reduction of 27%	[67]
Re, Jb, JbNO, Jl, Jz, DehydroJc, HydroxyJb, Eruc, Sk, Ot, Fl	Cheese	0.04% *v*/*v* mixed-strain mesophilic starter culture (composed of various strains of the species *Lactococcus lactis cremoris* and *Leuconostoc* spp.), Rennet of animal origin, 31 °C for 56 min	Reduction of 14%	[67]
Re, Sc, Jb, Sp, Im, He, Eu, Sk, ReNO, JbNO, SpNO, ScNO, ImNO, HeNO, EuNO	Black tea	Under high relative humidity and temperature for 5–6 h	Increase in the PA and PANO content	[72]
n.p. *[a]	Mead	n.p. *	Values found well above the average of regular retail honey	[86]
Ech, EchNO, Echi, EchiNO, AcetylEch, AcetylEchNO, AcetylEchi, AcetylEchiNO	Mead	n.p. *	Reduction of 30–70%	[87]
Tropane alkaloids				
An, Scp, At, Lt, Ap, Hm, Apo, Scpl, Tropi	Bread	37 °C during 1 h	Reduction of 19–65% (except for Ap, Apo and Tropi) [b]	[79]

* n.p.: not provided. [a] Pyrrolizidine alkaloids calculated as retronecine equivalents; [b] increase in the compounds during fermentation, possibly due to the degradation and transformation of other compounds into these molecules. Acetylechimidine (AcetylEch), acetylechimidine *N*-oxide (AcetylEchNO), acetylechiumine (AcetylEchi), acetylechiumine *N*-oxide (AcetylEchiNO), anisodamine (An), apoatropine (Apo), aposcopolamine (Ap), atropine (At), dehydrojacoline (DehydroJc), echimidine (Ech), echimidine *N*-oxide (EchNO), echiumine (Echi), echiumine *N*-oxide (EchiNO), erucifoline (Eruc), europine (Eu), europine *N*-oxide (EuNO), florosenine (Fl), heliotrine (He), heliotrine *N*-oxide (HeNO), homatropine (Hm), hydroxyjacobine (HydroxyJb), intermedine (Im), intermedine *N*-oxide (ImNO), jacobine (Jb), jacobine *N*-oxide (JbNO), jacobine *N*-oxide (JbNO), jacoline (Jl), jacozinec (Jz), littorine (Lt), otosenine (Ot), pyrrolizidine alkaloids (PAs), pyrrolizidine alkaloids *N*-oxide (PANOs), retrorsine (Re), retrorsine *N*-oxide (ReNO), scopolamine (Scp), scopoline (Scpl), senecionine (Sc), senecionine *N*-oxide (ScNO), seneciphylline (Sp), seneciphylline *N*-oxide (SpNO), senkirkine (Sk), and tropine (Tropi).

On the other hand, the effect of fermentation on TAs has only been evaluated by Marin-Sáez et al. (2019). The effect of fermentation (37 °C) was studied on bread prepared with flour contaminated with *Datura stramonium* and *Brugmansia arborea* [79]. Likewise, the same fermentation procedure was simulated with TA standard solutions [79]. The fermentation conditions showed great variability in the results, showing degradation levels of TAs between 16 and 95% in standard solutions subjected to fermentation conditions and between 19 and 65% in the contaminated bread samples. Nonetheless, in contrast, it was observed that the concentration of some TAs (aposcopolamine, apoatropine and tropine) increased after fermentation, such as in the case of baking [79]. Therefore, it is suggested that fermentation produces the degradation of high molecular weight TAs. Nonetheless, as in the case of PAs, more controlled studies are needed to understand the effect of fermentation on TAs.

Regarding OAs, to the best of our knowledge, there are no studies that address the effect of fermentation on the content of these alkaloids (Figure 3). Therefore, it would be interesting to carry out studies for these analytes in future work. In fact, many of the bakery products available in supermarkets have poppy seeds added as decorative toppings. Likewise, these seeds are increasingly been added to many doughs and yoghurts. Therefore, it would be interesting to see how these alkaloids are affected by the alcoholic and lactic fermentation processes.

3.3. Infusion

Most of the food alerts that have noted high concentrations of these alkaloids (e.g., PAs and TAs) have been reported in teas and herbal teas [6,7,9]. However, in many cases, the high levels of these alkaloids have been referred to the dry products instead of the hot drink obtained via the infusion process [88]. In this sense, many of the published works directly analyzed the dry samples instead of the infusions [88,89]. This is a relevant issue, as for the risk assessment of these contaminants it is also important to consider the transfer rate of these alkaloids in culinary preparations, such as infusions, so that a more reliable scenario of the real intake of these contaminants by consumers can be determined. Accordingly, besides thermal degradation, the investigation of the transfer rate of these alkaloids during the preparation of tea and herbal tea infusions with boiling water has also been one of the most studied processes, especially in the case of PAs (Figure 3). Table 3 collects the works available in the literature that have addressed this issue to date.

Currently, according to the EFSA, the concentration of PAs and PANOs in infusions is estimated by applying a dilution factor to the data obtained in the dry products considering a 100% transfer rate [89–92]. Nonetheless, current data in the literature concerning the transfer rate of PAs in infusion samples also show discrepancies. For instance, Rosemann (2007) evaluated the content of retrorsine in both dried and boiled herbal tea samples, observing no differences between both samples [71]. Mathon et al. (2014) also indicated the complete migration of PAs from the dry plant to the herbal infusion. The results obtained showed that the PA levels determined in hot tea drinks prepared with boiled water were similar to the PA concentrations quantified in the dry raw herbs after extraction under acidic conditions [93]. However, other authors have stated that the transfer rate of PAs from the dry product to the infusion was not always 100% [72,90,93–97]. Thus, some works have determined high transfer rates of PAs to infusions in the range of 80–100% [94–96]. For instance, Lüthy et al. (1980) indicated a transfer rate of 80% for senkirkine from coltsfoot [94], Mulder et al. (2018) reported about an 85% transfer rate for 28 PAs in 38 tea samples [95], and Engeli (2014) a range between 84 and 103% for nine PAs in peppermint infusions [96]. However, conversely, Picron et al. (2018) observed low transfer rates of PAs during the brewing process (16–28%, except monocrotaline with 45%) [90]. Similarly, Schulz et al. (2015) also found that the total PA content in infusion samples was relatively smaller than that of their respective dry herbal product, with concentration levels ranging between 13 and 1080 and 1127 and 5137 μg/kg, respectively [97].

Additionally, a high prevalence of PANOs was detected in aqueous infusion samples [97]. Likewise, this same trend was observed in the work of Han et al. (2022), in which the greater transfer rate of PANOs was attributed to their higher polarity [72]. However, in this case, higher transfer rates for PAs ($\leq$57%) and PANOs ($\geq$76%) were reported [72]. In the work of Reinhard and Zoller (2021), PANOs also showed higher transfer rates than PAs using different brewing processes [98]. In this sense, in samples prepared with the ISO brewing procedure [99], the transfer rates of PAs and PANOs were between 52 and 89% (median 73%) and 87 and 100% (median 91%), respectively [98]. Conversely, transfer rates of PAs and PANOs were higher in samples prepared according to the vendor's instructions, ranging between 63 and 95% (median 84%) and 95 and 100% (median 99%), respectively [98].

Therefore, PAs were extracted less efficiently with the ISO procedure than with the vendor's instructions. This fact highlights that there are many parameters that may affect the efficiency of the transfer rate during the brewing process of teas and infusions, such as the tea-to-water ratio, the brewing method (i.e., temperature, time, acidic conditions), the homogeneity of the tea samples (e.g., particle size of the leaves), or even the type/source of contamination (e.g., differences among the PA-producing plants, contamination by flowers, leaves, etc.). For instance, Chen et al. (2019) assessed the content of PAs and PANOs in infusions prepared with comminuted leaves and intact leaves [100]. The extraction efficiency was higher in the infusion prepared with comminuted leaves, showing PA concentration levels 1.1–4.1 times higher than in the infusions prepared with intact leaves [100]. Likewise, acidic conditions have shown to enhance the transfer rate of these alkaloids during the brewing process [98]. Nonetheless, it is unlikely that the pH of the brewed herbs would be low enough to have a significant effect on PA-extraction. Moreover, it seems that decreasing the tea-to-water ratio and increasing the steeping time contributes to increasing the transfer rate of PAs. Jansons et al. (2022) also compared the concentrations obtained for some PAs and PANOs in fennel and anise dry teas with the levels found of these alkaloids in their corresponding infusions (which were prepared following the instructions on the tea packaging) [70]. Although transfer rates were not provided by the authors, the results obtained showed a significant decrease in some PAs/PANOs in the hot drink (e.g., heliosurpine *N*-oxide, echinatine, europine and intermedine), suggesting a transfer rate lower than 100% [70]. However, the concentration level of other analytes, such as retrorsine *N*-oxide and senecionine *N*-oxide, were much higher in the drink than in the dry tea [70]. These higher concentrations led to greater amounts of PAs/PANOs in the infusions than in the dry extracts analyzed by QuEChERS. This has also been reported by other authors [95,98]. For instance, Mulder et al. (2018) determined a 2.4-fold content increase for infusions (aqueous medium at 100 °C) compared with aqueous acidic extraction at room temperature [95].

Therefore, the higher PA concentrations observed by some authors in hot tea drinks compared with the levels found in dry raw materials could be explained by the prolonged aqueous extraction at high temperatures, suggesting a lower extraction efficiency when other organic solvents in an acidic medium [70,95,98]. Hence, this fact is important when developing routine analytical methods to monitor the presence of these alkaloids, and thus legislation should contemplate these issues when establishing the maximum concentration limits in these types of product, as it seems to be more suitable to perform the analysis of infusions rather than of the dry product. In this sense, the analysis of infusions provides a more reliable scenario of the real intake and exposure of consumers to these alkaloids. However, due to the great variability of the data related to the transfer rate of PAs, more studies are needed to evaluate this phenomenon and carry out greater control of all the parameters that can influence it, so that a reliable PA transfer rate can be set. Likewise, it is important to determine if the transfer rate observed is associated to a degradation process or not. In some cases, the transfer rate can be lower because the compounds may be degraded by heat, while in other cases it may be lower due to a lower extraction efficiency. This aspect has not been discussed in any of the works reviewed. Moreover, it is also of high interest to determine if the lower transfer rate is due to a complete degradation of

the compound because of the heat applied or if a transformation into other compounds is taking place. As previously indicated in Section 3.1, if new compounds derived from PAs are being formed during the brewing process, they should be identify and assessed for their toxicity.

Regarding TAs, the transfer rate of TAs has scarcely been studied (Figure 3). In this sense, the EFSA evaluated the transfer rate of TAs from teas and herbal teas into infusion drinks, showing a transfer rate of about 50% [35]. However, great variability was found in the results achieved. Due to this fact, the EFSA recommended a more exhaustive study of the transfer rate of TAs in infusions and specifically, in children's infusions [35]. In this line, recent work of our research group evaluated the transfer rate of TAs in fennel samples finding a transfer rate between 64 and 88% for atropine and 47 and 57% for scopolamine [101]. Similarly, Marin-Sáez et al. (2019) confirmed the transfer rate of TAs to hot water in tea preparations. The results obtained showed a transfer rate between 20 and 92% from the plant to the tea water in all the assayed analytes [81]. Despite these works, more information is necessary to determine the transfer rate of TAs during the preparation of teas and herbal tea infusions. Likewise, it is important to evaluate other parameters that may influence the transfer rate of these alkaloids, such as the matrix, crushing of the sample, whether the product is fresh or dry, infusion temperature, infusion time, type of sample, etc. All of them should be carefully studied and, the determination of degradation products and their exposure through these types of products.

In the case of OAs, the consumption of poppy seed tea can become potentially dangerous, because consumers can add as many poppy seeds into the tea as they wish. Accordingly, some authors have evaluated the occurrence of OAs in some tea infusions prepared with poppy seeds [53,102–104], finding very high levels of OAs in some cases. For instance, Powers et al. (2018) stated that alkaloid yields varied among extractions, but regardless of the extraction conditions, lethal amounts of morphine were extracted from the poppy seed coats by homemade methods [53]. Therefore, the study of this type of sample is essential to highlight the dangers of this consumption practice and to warn the authorities of the need to control this type of product that may pose a potential risk to consumers' health. In addition, recent work by our research group evaluated the transfer rates of OAs from poppy seeds to teas during the brewing process. The transfer rates were less than 100% for some analytes, and confirmed that for some parameters, such as the infusion temperature and time, had a significant impact on the transfer rate [104]. Considering the results obtained, it would be interesting to confirm with further studies whether the lack of OA transfer from poppy seeds to the infusion is due to the thermal degradation of the OAs at high temperatures. Likewise, it is also important to consider the investigation of the presence of degradation compounds from OAs and their risk to the health of consumers.

3.4. Other Treatments (Grinding, Washing and Soaking)

There are other types of processes or pre-treatments that can be performed on foods, not only as a prior step before their processing or culinary preparation, but also to ease the elimination of some alkaloids, as in the case of OAs. Hence, the Recommendation (EC) 2014/662/EU suggests grinding as one type of processing that could be done to poppy seeds that are ingested crude (without the application of high temperatures) to reduce the OA content [82]. In this sense, grinding could result in a 25–34% reduction due to the accelerated degradation rate of morphine, resulting in large active surfaces, whereby oxygen-influenced pseudomorphine and morphine-*N*-oxide formation can occur in the product. In addition, this effect could be increased with oxygen and pH increases [82]. The effect of grinding was also evaluated in the work of Sproll et al. (2007), in which the degradation kinetics of morphine caused by grinding were studied [52]. The results obtained were in agreement with the degradation degree established in the EC Recommendation 2014/662/EU [52,82]. Furthermore, washing or soaking poppy seeds in water are culinary preparations that can also reduce the content of OAs [56].

Table 3. Transfer rate of pyrrolizidine, tropane and alkaloids during brewing processes according to literature data.

Analytes	Sample	Brewing Conditions	Transfer Rate	Reference
Pyrrolizidine alkaloids				
Ech, EchNO, En, EnNO, Eu, EuNO, Hs, HsNO, He, HeNO, In, InNO, Ig, IgNO, Im, ImNO, Ls, LsNO, Lyc, LycNO, Re, ReNO, Sc, ScNO, Sp, SpNO, Sv, SvNO, Sk, Us	Fennel and anise teas	n.p. *	n.p. * (lower than 100%) [a]	[70]
Re	Lucerne herbal tea	100 °C for 3 h	Approximately 100%	[71]
Re, Sc, Jb, Sp, Im, He, Eu, Sk, ReNO, ScNO, JbNO, SpNO, ImNO, HeNO, EuNO	Green tea	First infusion: 100 °C for 4 min Second infusion: 100 °C for 4 min	41–93%	[72]
Ech, Eruc, Eu, He, In, Im, Jb, Ls, Lyc, Mc, Re, Sc, Sp, Sv, Sk, Td, EchNO, ErucNO, EuNO, HeNO, InNO, ImNO, JbNO, LsNO, LycNO, McNO, ReNO, ScNO, SpNO, SvNO	Rooibos tea	100 °C for 6 min	16–45%	[90]
Ech, Lyc, Mc, Sc, ScNO, Sp, He, Ls, Sk, Re	Herbal teas (mix, rooibos, linden, mint, verbena, camomile)	100 °C for 10 min with slight agitation	Approximately 100%	[93]
Sk	Coltsfoot (*Tussilago farfara* L.)	n.p. *	80%	[94]
Re, Ls, LsNO, He, HeNO, Td, Mc, Sc, Sp, Ech, EchNO, Eruc, ErucNO, Eu, EuNO, Im, ImNO, Jb, JbNO, Lyc, LycNO, McNO, ReNO, ScNO, SpNO, Sv, SvNO, Sk	Teas (black tea, green tea) and herbal teas (rooibos, chamomile, peppermint, mix)	100 °C for 5 min	Overall, 85% (individual samples varied between 40 and 250%)	[95]
9 PAs	Peppermint herbal tea	n.p. *	84–103%	[96]
Ech, Eruc, ErucNO, Eu, EuNO, He, HeNO, Im, Jb, JbNO, Ls, LsNO, Lyc, Mc, McNO, Re, ReNO, Sc, ScNO, Sp, SpNO, Sk, Td	Teas and herbal teas (nettle, fennel fruits, chamomile, melissa, peppermint, mix (valerian, hopcone, lavender, caraway, anise, coriander))	100 °C for 15 min	1–21%	[97]

Table 3. *Cont.*

Analytes	Sample	Brewing Conditions	Transfer Rate	Reference
Ech, ErucNO, Eu, EuNO, Im, ImNO, Jb, JbNO, Ls, Lyc, LycNO, Mc, Sc, ScNO, Sk, Sv, SvNO, Td, Re, Sp, Jl, Hs, Rn, Ev, EchNO, HeNO, McNO, LsNO, ReNO, SpNO	Black tea, green tea, mixed herbal tea, peppermint tea, red bush tea, senna tea	ISO brewing procedure: 100 °C for 6 min (capping and shaking). The procedure was repeated two more times. Vendor's instructions for brewing: - Black tea: 100 °C for 4 min (uncapped) - Green tea: first extraction at 80 °C for 2 min (uncapped) + second extraction at 100 °C for 1 min (uncapped) - Mixed herbal tea: 100 °C for 10 min (capped) - Peppermint tea: 100 °C for 6 min (uncapped) - Red bush tea: 100 °C for 6 min (uncapped) - Senna tea: 80 °C for 10 min (uncapped)	52–100% (ISO brewing procedure) 63–100% (vendor's instructions)	[98]
Tropane alkaloids				
At, Scp	Tea	100 °C for 4.5 min	42–54%	[35]
An, Scp, At, Lt, Ap, Hm, Apo, Scpl, Tropi	Tea	Tea: 100 °C, left to cool for 5 min	20–92%	[81]
At, Scp	Fennel tea	100 °C for 5 min	47–88%	[101]
Opium alkaloids				
Morphine (Mor), Codeine (Cod), Thebaine (Th) Papaverine (Pap), Noscapine (Nos)	Poppy seed tea	90 °C for 5 min	71–100%	[104]

* n.p.: not provided. [a] Except ReNo and ScNO concentrations, which were much higher in the drink than in the dry tea, probably due to different extraction conditions (solvents and temperature). Anisodamine (An), apoatropine (Apo), aposcopolamine (Ap), atropine (At), codeine (Cod), echimidine (Ech), echimidine *N*-oxide (EchNO), echinatine (En), echinatine *N*-oxide (EnNO), echivulgarine (Ev), erucifoline (Eruc), erucifoline *N*-oxide (ErucNO), europine (Eu), europine *N*-oxide (EuNO), heliosupine (Hs), heliosupine *N*-oxide (HsNO), heliotrine (He), heliotrine *N*-oxide (HeNO), homatropine (Hm), indicine (In), indicine *N*-oxide (InNO), integerrimine (Ig), integerrimine *N*-oxide (IgNO), intermedine (Im), intermedine *N*-oxide (ImNO), jacobine (Jb), jacobine *N*-oxide (JbNO), jacobine *N*-oxide (JbNO), jacoline (Jl), lasiocarpine (Ls), lasiocarpine *N*-oxide (LsNO), littorine (Lt), lycopsamine (Lyc), lycopsamine *N*-oxide (LycNO), monocrotaline (Mc), monocrotaline *N*-oxide (McNO), morphine (Mor), noscapine (Nos), papaverine (Pap), pyrrolizidine alkaloids (PAs), rinderine (Rn) retrorsine (Re), retrorsine *N*-oxide (ReNO), scopolamine (Scp), scopoline (Scpl), senecionine (Sc), senecionine *N*-oxide (ScNO), seneciphylline (Sp), seneciphylline *N*-oxide (SpNO), senecivernine (Sv), senecivernine (SvNO), senkirkine (Sk), thebaine (Th), trichodesmine (Td), tropine (Tropi), and usaramine (Us).

According to Recommendation (EC) 2014/662/EU [82], washing for 5 min can reduce the content of OAs by 46%. In fact, this reduction can be higher by increasing the washing time and washing temperature. In this sense, a 100% reduction can be reached at 100 °C for 30 min. In addition, a single washing in slightly acidic conditions can also reduce the content of OAs by up to 40%. Therefore, the labelling of poppy seeds should indicate the pre-treatment of the seeds and recommendations for their use, as this could be useful both for the consumers themselves and for manufacturers of bakery products. However, the effect of these procedures has been little reported and only in the case of OAs (Figure 3). Nonetheless, it could be interesting to carry out more studies concerning the effect of these preparations on the different types of alkaloids in future work. For instance, TAs and PAs (mainly PANOs) are very water-soluble compounds, so a washing or soaking step may also reduce their content, such as in the case of vegetables or legumes contaminated with these alkaloids by natural horizontal transfer. Likewise, the grinding performed on spices and aromatic herbs may also have an influence on the content of these alkaloids.

4. Conclusions

The effect of food processing and culinary preparation on the content and stability of natural toxins, such as PAs, TAs and OAs, is a research field that, to date, has been scarcely studied, since there are not many works published addressing this issue. However, the works that do exist show that this research topic is increasingly booming and that they constitute a research line of great interest for future work with many challenges to be resolved.

Accordingly, to date, the effect of thermal treatments on the concentration of PAs, TAs and OAs has been well studied, including the investigation of the infusion rate of these compounds during the preparation of tea and herbal tea infusions with boiling water. Currently, knowledge about the effect that thermal treatments have on the content of these alkaloids, including the detection of their transformation products, is very limited and inconclusive. One of the reasons for the great controversy of results is the lack of well-designed studies, with exhaustive controls of all the variables involved in the process. In fact, almost all the works addressing this issue did not provide kinetic or thermodynamic data related to the thermal degradation process of these alkaloids. These studies are necessary to perform a more real assessment of the population's exposure to these toxic alkaloids. Currently, in the case of PAs, it seems that thermal treatments have a more pronounced effect in the stability of PANOs, while PAs are more stable compounds. TAs and OAs also seem to have a certain sensitivity to heat, as reductions in their content have been reported at high temperatures. Nonetheless, to date there are no conclusive findings, and it is worth considering that the reduction observed in these compounds in many cases is due to their interconversion or transformation into other products. Accordingly, PANOs can also be degraded by heat into their corresponding free base primary PAs, maintaining the total levels of PAs. Likewise, complex TAs, such as atropine and scopolamine, can be degraded to low molecular weight TAs or other degradation products (e.g., aposcopolamine and apoatropine) which are even more toxic. Moreover, another relevant point which is worth considering is the TA chirality issue, as some works have described racemization processes due to heat. Therefore, it is not only important to determine the stability of these alkaloids, but also to evaluate the production of transformation products and evaluate their toxicity. In fact, if toxic degradation products are formed, they should also be considered in the legislation of these alkaloids to establish maximum concentration limits for them to ensure the health of consumers.

On the other hand, the effect of fermentation has been studied to a lesser extent, and only in the case of TAs and PAs, more so in the latter. It seems that fermentation may contribute to reducing the content of these alkaloids due to microbial activity; however, due to the limited available data more studies are needed to draw reliable conclusions and to understand the microbial action mechanism on these alkaloids.

Regarding the transfer rate of these alkaloids, it seems that many parameters may influence the process. Moreover, the published works reveal that the analysis of infusions is most suitable than the dried products to avoid an overestimation of the real intake and exposure of consumers to these alkaloids. Moreover, it has been highlighted that to determine the content of these alkaloids it is preferably to prepare infusions according to the vendor's instructions in order to assess realistic consumer exposures. Accordingly, the limits set in the legislation should be revised considering these aspects.

Finally, the effect of grinding, washing and soaking has been little reported and only in the case of OAs, so it could be interesting in future work to carry out more studies concerning the effect of these preparations on the different types of alkaloids.

Overall, it can be concluded that works dealing with the effect of food processing and culinary preparation on these alkaloids are very limited, and the few available results are very uncertain in all the treatments reviewed. For this reason, it is of high interest to carry out well-designed future studies that delve deeper into the subject, focusing on the adequate control of critical variables and parameters that can affect the stability of these compounds (e.g., temperature, time, degree of contamination, source of contamination, type of matrix, etc.). Likewise, the investigation of the transformation of these alkaloids into toxic degradation products is also of high interest and should be addressed in works within this research field.

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