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Opium alkaloids in food products: current and future perspectives

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Abstract:	Background: In recent years, there has been increasing interest from health authorities in avoiding consumer exposure to opium alkaloids in food. Thus, recent cases of intoxication and false positive drug tests, from the consumption of poppy seeds and food, have been detected. In order to know more certainly the concentration of these substances in food and to establish more reliably the consumption of these toxics in the population, data on their presence in food should be further collected. These compounds are found at ultra-trace levels in complex matrices, so it is important to develop efficient analytical methods based on selective analytical techniques and adequate sample treatment, which is key to avoid matrix effects. Scope and approach: This review summarizes the actual situation of opioids in food products. It establishes the cause of their presence in food, the risk of consumption and actions to prevent their exposure. In addition, it sums the techniques of sample treatment and analysis of all available articles on opioids in different samples. Key findings and conclusions: The studies that have been made of opioids are mainly about morphine. For this reason, there is a need to do more studies with all of them. Besides, most of the studies are in biological samples, following consumption of poppy seeds or foods. Therefore, there is to develop and validate new methods that are effective for complex matrices such are foods, to know exactly the actual exposure to consumers and how to decrease it.
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Dear Editor,

Please find enclosed the manuscript entitled "**Opium alkaloids in food products: current and future perspectives"** that we wish to publish as a full paper in the *Trends in Food Science and Technology* journal. This review is unpublished and is not being considered for publication elsewhere. The total number of tables is 3 and the total number of figures is 4.

With best regards,

Dr. Isabel Sierra

Highlights

- ► Occurrence of opium alkaloid in poppy seeds and food elaborated with poppy seeds
- ► Problems caused by consumption of food products contaminated with opioids
- ► Actions to avoid opioids in food products
- ► Analytical methods for the determination of opioids in poppy seeds and food products

ABSTRACT

Background: In recent years, there has been increasing interest from health authorities in avoiding consumer exposure to opium alkaloids in food. Thus, recent cases of intoxication and false positive drug tests, from the consumption of poppy seeds and food, have been detected. In order to know more certainly the concentration of these substances in food and to establish more reliably the consumption of these toxics in the population, data on their presence in food should be further collected. These compounds are found at ultra-trace levels in complex matrices, so it is important to develop efficient analytical methods based on selective analytical techniques and adequate sample treatment, which is key to avoid matrix effects.

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Opium alkaloids in food products: current and future perspectives

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Keywords: Opium alkaloids; Food products; Poppy seeds; Papaver plant; Analysis technique; Sample treatment

1. Introduction

Opium poppy seeds are quite popular in many counties of the world as a food ingredient or to make tea. These seeds do not contain opium alkaloids, but poor harvesting practices or insect damage can contaminate them with latex, which is rich in opium alkaloids (AESAN, Spanish Food Safety and Nutrition Agency, 2016). Consumption of these alkaloids may involve several risks, especially for the most vulnerable people**.** Furthermore, some cases of intoxication and false positive drug tests have also been reported (Lachenmeier, Sproll & Musshoff, 2010; Sproll, Perz, & Lachenmeier, 2006).

Therefore, many countries have taken measures, such as establishing for food use papaver plant varieties with a low level of alkaloids, setting maximum limits or, even, prohibiting their use for food (BfR, German Federal Institute for Risk Assessment, 2006; EFSA, European Food Safety Authority, 2011). However, there is no common legislation, which reduces their control and makes marketing between different countries more difficult (AESAN, 2016). For this reason, the health authorities want to establish harmonised legislation, but to do this, it is needed to know the real exposure of all these toxins in people (EFSA, 2018). So far, studies are mainly focused on morphine, but all the opium alkaloids that can be found in poppy seeds should be taken into account. Therefore, it is important to carry out studies about the content of opium alkaloids in poppy seeds and in food products containing poppy seed, which are being commercialized nowadays.

So far, numerous studies that have been done are on biological samples, following the consumption of seeds and poppy seed foods. Positive results have been obtained demonstrating the presence of considerable amounts of opioids in these samples (Moeller,

Hammer & Engel, 2004; Newmeyer et al., 2015; Özbunar et al., 2019). However, there are very few studies on food matrices, so research is required to develop and validate new analytical methods to quantify these compounds in food products. As they are found in very low concentrations in these complex matrices, it is necessary to use analytical methods involving selective techniques and adequate sample treatment. Concerning sample treatment, in recent years there has been increasing interest in exploring new techniques that extend beyond simple extraction of the target analytes with organic solvents because this technique is laborious and requires a high amount of biologically toxic solvents. The use of more innovative and selective techniques has been increased, which allow the purification of the sample extract, thus avoiding matrix effects, especially in food matrices. For these compounds, the most remarkable is the solid-phase extraction (SPE) with conventional commercial sorbents (Özbunar et al., 2019). However, current trends in sample preparation involve moving towards "greener" approaches by scaling down analytical operations and integrating new advanced materials as sorbents. For other types of natural toxins, much progress has been made in the application of new materials in the purification stage and their integration in micro-extraction techniques (Casado, Gañán, Morante-Zarcero & Sierra, 2020). Unfortunately, as far as we know, only a recent study has been published for the analysis of opium alkaloids in foods, where miniaturization in sample preparation has been applied (Xu, Liu, Wang & Wei, 2019).

The aim of the present review is to compile all the information available on the presence of opioids in food products, to make a generalist analysis of the situation of this family of toxins today (problems involved in their consumption and solutions that are being carried out) including future research perspectives in this field (Fig. 1).

2. Origin of opium alkaloids in food products

Opium poppy (*Papaver somniferum* L.) is a member of the Papaveraceae family, which has been known since ancient times as a medicinal and culinary plant for its pharmaceutical and nutritional properties.

On the one hand, its milky latex sap (opium) obtained from the capsules contain opium alkaloids. So far, around 50 different alkaloids have been isolated from opium and morphine is present in the largest concentration. Fig. 1S (supplementary material) shows the chemical structure of the principal alkaloids from opium poppy, such as morphine, codeine, thebaine, papaverine, noscapine, narceine and oripavine. These alkaloids are of considerable importance in medicine and pharmacy for their analgesic properties, coronary vasodilator function, potentially anti-cancer drug and cough suppressant features. For this reason, this traditional plant is widely used as a medicinal plant to treat cramp, chronic lax and chronic cough (Labanca, Ovesnà, & Milella, 2018).

On the other hand, poppy seeds are used in food processing because of their good nutritional quality, since they are generally rich in fatty acids (Özcan & Atalay, 2006), predominantly linoleic, oleic and palmitic acids (Ghafoor, Özcan, AL-Juhaimi, Babiker & Fadimu, 2019). For this reason, poppy seeds are increasingly used in Central Europe, such as bakery products, bean-jam buns, toppings for dishes, in fillings of cakes and desserts and to produce edible oil (AESAN, 2018). Another widespread use of poppy seeds is in making tea that helps to relax and sleep (Haber, Pergolizzi & LeQuang, 2019; Powers, Swortwood & Erickson, 2018). In addition, these seeds are sold alone or mixed with other seeds, such as chia or flax, and are commonly added for example to salads or purees, and in ground form are used as flavouring ingredient of pasta. Other recent industrial uses of these seeds are the preparation of dairy products (yoghurts) and snacks (AESAN, 2016).

There are poppy plants with different concentrations of opium alkaloids because alkaloid accumulation depends on several factors. The most influential are genetic factors and environmental conditions (EFSA, 2018). For example, Meos, Saks and Raal (2017) determined the morphine content in dried capsules of 34 different cultivars grown in Estonia, finding a broad range for this alkaloid between 0.57 and 6.76 g kg^{-1}. Hence, it is important to establish a classification according to the content of opioid alkaloids, differentiating types with high opioid content from those with low concentrations. Theoretically, varieties that have high morphine levels (> 0.8%) are used in pharmacy and varieties with fewer morphine levels are used in food. However, some varieties have low morphine level, but a high level in other opium alkaloids, for example, papaverine (Stranska, Skalicky, Novak, Matyasova & Hejnak, 2013). For this reason, it is necessary to consider the quantity of all opium alkaloids. In addition, poppy seeds from some *P. somniferum* varieties with high alkaloid content, specially grown for pharmaceutical applications, are sometimes used as a sub-product for food use (EFSA, 2018). This is because no harmonised European legislation has yet been created on this family of toxins in poppy seeds for food purposes.

Besides, it is important to highlight that unlike latex, seeds hardly contain any opium alkaloids, so it was thought that they could be used freely for food processing. However, in the last few years have seen some cases of intoxication, false positive drug tests and dependencies. For this reason, some scientists have tested biological samples, such as blood, urine or oral fluid after consumption of poppy seeds or food products containing poppy seeds. After that, they have shown that the consumption of these can give considerable amounts of opium alkaloids in biological samples. This was already demonstrated in 1982 by Bjerver, Jonsson, Nilsson, Schuberth and Schuberth in a study where healthy subjects that consumed one or two rations of poppy seed cake presented a

147 significant content of morphine in the urine. In another study, Hayes, Krasselt and
148 Mueggler (1987) get considerable amounts of morphine and codeine in serum and urine
149 following ingestion of poppy seeds. These results can be attributed to the fact that poppy
150 seeds have considerable amounts of opium alkaloids due to contamination with the latex.
151 This contamination may be due to poor harvesting practices or insect damage.
152 Consequently, it is important to keep track of the conditions of the crop, in order to use
153 poppy seeds in the preparation of food products (AESAN, 2016).
154

155 ## 3. Opium alkaloid content in poppy seeds and food products

156

157 Table 1 collects the content of six opium alkaloids (morphine, codeine, thebaine,
158 papaverine, noscapine and narceine) that have been analysed in poppy seeds, poppy seed
159 foods and poppy teas. The objective, in many of these studies, was to know the presence
160 of the alkaloids in blood, oral fluid or urine after consumption of these products.
161 Therefore, in order to carry out a controlled administration of these opioid alkaloids to
162 the subjects, its concentration in the poppy seeds, foods and teas were previously
163 analysed. In some other works, the objective was to determine if the concentration of
164 opium alkaloids changes during food processing. For this reason, in parallel to
165 establishing the concentration of these alkaloids in seeds, the levels of opioids in different
166 food products prepared from poppy seeds were also analysed.

167 In poppy seeds, significant amounts of opium alkaloids have reported in some studies
168 (Table 1). For example, Bjerver et al. (1982) determined morphine in 5 poppy seeds (3
169 blue and 2 white seeds). They got considerable amounts of morphine, specially in two
170 blue varieties (85.6 and 106.7 mg kg^{-1}). Hayes et al. (1987) found morphine and codeine
171 in four samples of black poppy seeds in the range of 17 - 294 mg kg^{-1} and 3 - 14 mg kg^{-1},

respectively. In seeds from Australia, Casella et al. (1997) found 164 mg kg^{-1} of morphine, 31.8 mg kg^{-1} of codeine and 20.7 mg $kg^{-1}$of thebaine, whereas in seeds purchased in a shop in Manchester similar values were observed for morphine, but less than half for codeine and thebaine (Table 1). On the other hand, in some other works lower amounts of these compounds have been found. This is the case of Meadway, George and Braithwaite (1998), who reported 0.6 - 11.9 mg kg^{-1} for morphine and 0.3 - 0.7 mg kg^{-1} for codeine in 4 samples of blue and 1 sample of white poppy seeds from Australia, The Netherlands and Turkey. In some other studies, a high number of poppy seed samples have been analysed. Thus, Sproll et al. (2006) determined the opium alkaloid content of 83 different samples. Morphine was the main alkaloid in all of them (< 1 - 270 mg kg^{-1}), codeine was between < 0.3 - 56 mg kg^{-1}, and noscapine and papaverine were detected in isolated cases (mostly below the quantification limit). More recently, López et al. (2018) determined the levels of opium alkaloids in samples acquired in the Netherlands, Germany and Italy (33 blue, 3 white and 3 ground blue poppy seeds). All analysed samples contained morphine, in a range between 0.2 - 241 mg kg^{-1}. Codeine (< 0.1 - 348 mg kg^{-1}) and thebaine (< 0.1 - 106 mg kg^{-1}) were found in 78% of the samples. Lower amounts of noscapine (< 0.1 - 6 mg kg^{-1}), narceine (< 0.1 - 2.1 mg kg^{-1}) and papaverine (< 0.1 - 3.8 mg kg^{-1}) were also quantified in 85%, 46% and 37 % of the samples, respectively. In addition, in this study, some different opium alkaloid profiles could be identified depending on the origin of the samples. Thus, as can be seen from these works, the amount of opium alkaloids in poppy seeds is very variable. The poppy variety is an important influential factor, besides the geographical origin, the time of harvest and the external contaminations that can be produced during its recollection (López et al., 2018; Sproll et al., 2006).

196 The processing method for food containing poppy seeds is another important variable
197 to take into account. In this sense, as can be seen in Table 1, a significantly lower amount
198 of opium alkaloids in poppy seed foods is observed. In baking mixes containing poppy
199 seeds (Sproll et al., 2006), poppy seed filling for bakery (Pettitt, Dyszel & Hood, 1987;
200 López et al., 2018) and pastes from white, yellow and blue-black poppy seeds from
201 Turkey (Özbunar et al., 2019) low levels of morphine and codeine were detected. This
202 fact can be attributed to the production procedure of these mixtures where washing,
203 soaking with water, heating, grinding and/or crushing can be applied. In the same way,
204 Yamaguchi, Hayashida, Hayakawa, Nihira and Ohno (2011) determined opiates in bean-
205 jam buns decorated with poppy seeds. The morphine and codeine concentrations obtained
206 were 2.20 and 0.77 µg per one piece of bun, respectively. A similar situation was observed
207 in two ready-to-eat bakery products containing poppy seeds, where 0.6 mg kg^{-1} morphine
208 and 0.1 mg kg^{-1} thebaine, in one of them, and 0.5 mg kg^{-1} morphine and 0.2 mg kg^{-1}
209 noscapine, in the other, was observed (López et al., 2018). For this reason, these authors
210 concluded that there was a significant reduction in opium alkaloid content after heat
211 processing.
212 In recent years, the addition of poppy husks (pericarpium papaveris) containing opium
213 alkaloids to some food seasoning has been reported. This illegal use, motivated by
214 economic interests because of the dependence that its taste can cause, has been applied
215 for the preparation of hot pots. For this reason, some works have studied the presence of
216 opium alkaloids in these types of samples. One of them was Guo, Zhang, Zhao and Shao
217 (2013) who analysed 29 hot pot broth samples and determined three positives: one
218 contained with noscapine (0.22 µg kg^{-1}) and papaverine (0.16 µg kg^{-1}) and the two others
219 with morphine (22.5 and 28.9 µg kg^{-1}). More recently, Xu et al. (2019) analysed 30 hot

pot samples, but they showed concentrations below the detection limit (0.05-0.8 µg kg^{-1}) in all cases.

Finally, another popular use of poppy (seeds and plant) is in the preparation of teas. Van Thuyne, Van Eenoo and Delbeke (2003) analysed the morphine content in two herbal infusions, containing parts from the papaver plant among other herbals, obtaining 10.4 mg kg^{-1} in the first one, and 31.5 mg kg^{-1} in the second one. In the same way, Powers et al. (2017) studied opium alkaloids in teas prepared with bulk poppy seeds, poppy seed powder, poppy seed tea bag and liquid poppy extract. For this task, they prepared teas with four home-brewing methods (room temperature and heated water, with and without an acid modifier). Alkaloid yield varied between extractions, and under the best conditions concentrations in the range < 1 - 2788 mg kg^{-1} for morphine (heated neutral), < 1 - 247.6 mg kg^{-1} for codeine (heated acidic) and < 1 - 124 mg kg^{-1} for thebaine (heated acidic) were observed. Consequently, it was concluded that high levels of opium alkaloid could be found in these preparations and hence they could be potentially harmful.

According to all of these studies, in the evaluation of opium alkaloids in poppy seeds, it should be considered its posterior use in food. It is not the same to use the seeds to prepare bakery products, which involves a baking process, than to use them to make tea, or, as it is very common nowadays, to use them as a dressing in yoghurts or salads, which do not involve any previous treatment. According to Zentai, Sali, Szeitzné-Szabó, Szabó and Ambrus (2012) who evaluated the consumption pattern of poppy seeds in 2009 in Hungary, the consumption of raw and ground poppy seeds is higher (65%) than baked poppy seeds (35%). This is one more reason to control this family of contaminants and prevent them from the consumer. Unfortunately, there are very few studies done in food samples, so it is difficult to establish the real exposure of opium alkaloids in the population from the consumption of food products based on poppy seeds.

4. Problems of their presence in food

According to EFSA, for every approximately 100 g of cake or bun the amount of seeds used varies between 3.8 and 41 g (near half of the product), with an average content of 14 g (EFSA, 2011). Despite being a low amount, in some cases, it is demonstrated that the consumption of poppy seeds contaminated with opium alkaloids can lead to adverse health effects, especially in babies, infants, the elderly and people with severe health issues. For example, their consumption can lead to light-headedness and enteroparesis (BfR, 2006).

Older articles from the 19th century already reported cases of child intoxication with opium poppy. However, in recent years there have been a few more cases. Thus, King, McDonough, Drummer & Berkovic (1997) reported the case of a 26-year-old baker who after drinking poppy seed tea went to the hospital for hallucinations and showed a high morphine content in his blood and urine. Agin, Calkavur, Özdemir and Bak (2003) published the case of a child intoxication in Turkey due to the ingestion of the boiled poppy plant. Another case of intoxication is the one published by Hahn et al. (2008), in which they reported a serious health impairment of a 6-week-old baby associated with the ingestion of the boiled poppy seeds.

Another point to note is that opiates are included in the federally mandated workplace and DUID (driving under the influence of drugs) testing programs because of their psychoactive properties and frequently abuse as illicit drugs, about 1.3 million approximately of consumers in Europe (Rosado, Barroso, Vieira & Gallardo, 2019). In addition, the universally accepted cut-off limit of 300 ng mL^{-1} for opiate testing, declared mandatory for all drug testing laboratories by the Substance Abuse and Mental Health

Services Administration, has recently been questioned because the consumption of poppy seeds or products containing them without any illicit reason can cause false positive tests. For this reason, several studies measured opium alkaloids content in urine after ingestion of food products since the 1980s. An example is a study by Struempler (1987) that determined codeine and morphine concentrations in urine in different healthy individuals after ingestion of poppy seed bagels. Lo and Chua (1992) also determined these two opium alkaloids in urine after ingestion of curry meal. Meadway et al. (1998) and Yamaguchi et al. (2011) saw positive results in urine after ingesting poppy seed products (cakes and bean-jam buns). Also, there are different works in which it has been shown that positive results are obtained in other biological matrices (blood, plasma or oral fluid) following ingestion of poppy seeds (Moeller et al., 2004; Özbunar et al., 2019; Rohrig & Moore, 2003; Smith et al., 2014; Newmeyer et al., 2015). Therefore, all these previous studies show that a positive finding of any opium alkaloid does not necessarily mean an illicit drug use. For this reason, there is concern about interpreting the data produced when examining opiates. To avoid misunderstandings, professional athletes were advised not to take poppy seeds in their food or tea, as they are responsible for the appearance of doping substances in their biological samples (Van Thuyne et al., 2003). All this has attracted the interest of some researchers in determining biomarkers to differentiate whether the positive result is a result of illicit drug use or the ingestion unintentionally of food with poppy seeds. However, this is not an easy aspect and therefore there is some controversy. Cassella, Wu, Shaw and Hill (1997) and Meadway et al. (1998) suggested thebaine as a marker for culinary use because this type of opium alkaloid present in poppy seeds is not in drugs or in urine of real opiate drug users. Other authors argue that thebaine elimination varies significantly from one person to another, so its absence in a biological sample is not necessarily indicative of illicit drug

use. Therefore, at present, some studies preferred to take more caution and choose to consider thebaine only as a supportive biomarker (Özbunar et al., 2019). On the other hand, Trafkowski, Madea and Musshoff (2006) published that noscapine and papaverine could be used cautiously as additional markers for illicit heroin abuse because these types of opium alkaloids have not been detected following oral ingestion of opium poppy seeds at normal doses. However, this is not completely true, as noscapine and papaverine could be present in unusually high amounts and thus give a positive result in the urine of abusers. Alternatively, Yamaguchi et al. (2011) published that, concentrations of free morphine and codeine in urine can become indicators to differentiate whether detected opiates are subsequent to the consumption of food with opium poppy seeds or are due to the abuse of opiates. In summary, more research is needed on the possible markers to be used because there are some ideas, but no evidence yet that can differentiate these two very different uses.

5. Solutions

5.1 Legislation

Nowadays, there is no common legislation, and the situation in each country is different. Some countries have banned the use of poppy seeds for food applications. An example of this is China and some other Asian countries, where the government decided to ban the consumption of this plant. This is because some illegal traders were using pericarpium papaveris as a food additive to attract more customers, in particular using it in the hot pot, which is one of the most popular dishes in Chinese restaurants (Guo et al.,

319 2013). Another example is Belgium, where the use of poppy seed was banned in all foods,
320 except bakery products (EFSA, 2011).

321 In general, the use of poppy seeds in food is not prohibited in Europe. This is because
322 *P. somniferum* cultivars used for culinary purposes generally have low or moderate
323 morphine concentrations. However, no common legislation has yet been established to
324 differentiate between varieties with a high opium alkaloid content, and therefore only
325 suitable for pharmaceutical use, and those which can be used in foodstuffs because of
326 their low content. In addition, another aspect to be taken into account is that poppy seeds
327 with high opioid alkaloid content intended for gardeners as ornamental poppies are
328 commonly found on the market, which leads to the uncertainty of whether the seeds of
329 these plants are used for human consumption. For this reason, unlike the rest of Europe,
330 according to BfR (2006), there are low-morphine varieties certified for cultivation in
331 Germany ("Przemko") and Austria (“Edel-Weiß", "Edel-Rot", "Florian", "Josef", "Zero"
332 and "Zero 2000"). Furthermore, some authors are researching in low-morphine content
333 varieties, with the aim to establish cultivars for food consumption (Németh and Bernáth,
334 2009).

335 Despite the problems involved in the consumption of contaminated poppy seeds, as
336 they are often consumed in small doses, it is not surprising that harmonised legislation
337 has not yet been made in Europe, to establish the maximum limits for opium alkaloid in
338 poppy seeds or food with poppy seeds. However, the fact that poppy seed consumption
339 is low may be questionable, as there are countries with a longer tradition than others. In
340 Turkey, one of the main legal poppies producing countries in the world, they have a very
341 widespread use of this product and can even use more than 100 g of poppy seeds in one
342 food (Özbunar et al., 2019). In that respect, some countries have established maximum
343 limits, and the BfR established a provisional reference value of 4 mg kg^{-1} of morphine in

poppy seed for use in food, although this value is a limit of action that has no legislative purpose. Furthermore, Hungary has national maximum levels in poppy seeds of 30 mg kg^{-1} for morphine, 20 mg kg^{-1} for noscapine, 40 mg kg^{-1} for the sum of morphine and noscapine, 20 mg kg^{-1} for thebaine and 20 mg kg^{-1} for codeine (EFSA, 2011).

The fact that the situation is different in each country of European Union (EU) has generated numerous alert notifications in the RASFF (Rapid Alert System for Food and Feed), specifically 30 notifications since the first one in 2005 as shown in Table S1 (see supplementary material). All notifications were in seeds of different poppy varieties and origin, except one alert in 2019, which was in frozen bread. Besides, it should be noted that most of the notifications were based on the high morphine content. Only from 2015 notifications included other morphine alkaloids, but it was not specified which of them. As shown in Fig. 2, the high number of notifications generated since the beginning of 2019, compared to previous years is remarkable. This may be because other opium alkaloids started to be considered, as of the nine notifications that have been generated since 2019, seven include the other opium alkaloids. On the other hand, to prevent these products contaminated with high concentrations of opium alkaloids from reaching the consumer, several actions have been taken (Table S1). The action mostly carried out is the withdrawal of the product (57% of the cases). Other actions where prohibition to trade - sales ban (10%), official detention (7%), re-dispatch (7%) or even destruction (3%). In short, as there is no common legislation, a large part of the alert notifications is created from the export of seeds, from one country to another, which has more restrictive regulations. This situation jeopardises the viability of the single market as seed produced in one Member State cannot be marketed in another (AESAN, 2016).

EFSA has published some scientific opinions on the public health risks from the presence of opium alkaloids in poppy seeds used for human consumption. The first was

in 2011, prepared by the Expert Panel on Contaminants in the Food Chain (CONTAM Panel) which calculated an acute reference dose (ARfD) of 10 µg morphine per kg body weight (b.w.). This was an agreement between the different Member States of the EU in November 2016, which despite not being obligatory, involves their compromise and acceptation (AESAN, 2018). They considered that this amount represented the intake level above which foods with poppy seeds contaminated with opium alkaloid could be a health issue. It was established following a risk assessment, in which Germany, Hungary, Austria and the Netherlands indicated that morphine was the major alkaloid in poppy seed samples at concentrations up to 630 mg kg^{-1}. However, the conclusion published by EFSA was that more data needed to be collected on the presence of opium alkaloids other than morphine, such as codeine, papaverine, thebaine, noscapine and oripavine, in order to be able to perform the risk assessment more accurately (EFSA, 2011). A second opinion of the CONTAM Panel (EFSA, 2018) confirmed the ARfD of 10 µg of morphine per kg b.w. and further established that the concentration of codeine in poppy seed samples should be calculated in morphine equivalents, using a factor of 0.2. Therefore, the ARfD is the sum of morphine and codeine, expressed in morphine equivalents. Until 2018, risk assessments were mainly based on morphine and codeine, while other opium alkaloids remained unassessed due to lack of data. It was suggested that these other alkaloids should not be underestimated, and considered less risky than morphine or codeine, since thebaine has been shown to have a higher acute lethality and the estimated exposure could present a health risk (Eisenreich et al., 2020). The situation since then has not yet been resolved and the absence of available data on other opium alkaloids means that further studies are still needed to carry out a successful hazard characterization.

5.2 Recommendations for reducing opium alkaloids in poppy seeds

Another aspect to emphasize is that the problem of contamination of poppy seeds with the alkaloids contained in poppy latex could probably be solved by using less aggressive harvesting methods (BfR, 2006). For example, Moeller et al (2004) reported that the harvesting method used in low wage countries, where capsules are still opened manually and seeds are collected in a container, results in less contamination of the seeds with the milky sap. Consequently, the European Commission (2014) published a set of recommendations for good agricultural practices to avoid the presence of opiate alkaloids during cultivation, harvest and storage. For cultivation, it is recommended to choose varieties with lower content of opium alkaloids for food use, to control possible fungal diseases and pests and to use growth regulators to avoid lodging. During harvesting and storage, it is recommended to control the percentage of humidity.

On the other hand, summarizing the research that had been done on this subject, it can be also recommended some processing practices to avoid (or reduce) the presence of opium alkaloids in seeds and foods with poppy seeds (Table 2). The most efficient method that was first discovered was washing or soaking with water the poppy seeds, that can reduce about 40-75% (Bjerver et al., 1982; Lo & Chua, 1992). Sproll, Perz, Buschmann and Lachenmeier (2007) found that if hot water was used, it would still get a 100% reduction. In addition, by grinding the seeds it can be possible to get a moderate reduction (25-35%) in morphine content (Sproll et al., 2007; Zenai et al., 2012). This is a very important concept for the elaboration of foods that carry crude poppy seeds as toppings. Additionally, it has also been demonstrated that the combination of poppy seed pre-treatment (washing) with food preparation (baking) results in an overall reduction of 80-100% in the final food product (Lachenmeier et al., 2010). In summary, it seems that by

418 adequate treatment of the seeds, most morphine content is eliminated. However, more
419 studies are needed to know how these type of treatments affects the other opioid alkaloids.
420
421 *5.3 Analysis methods*
422
423 Table 3 collects different analytical methodologies that have been used in the
424 literature (from 1982 until 2020) to determine opium alkaloids in poppy (seeds and plant),
425 foods with poppy seeds and teas. As biological samples are out of the scope of this review,
426 methods for opium alkaloids determination in these samples (blood, serum, urine, oral
427 fluid) will not be discussed in this section. This information is presented in Table S2
428 (supplementary material).
429
430 *5.3.1 Analytical techniques used for detection and quantification of opium alkaloids*
431 There are many suitable analytical techniques for identifying and quantifying opium
432 alkaloids (Fig. 3), such as gas chromatography (GC), high-performance or ultra-high
433 performance liquid chromatography (HPLC, UHPLC) and capillary electrophoresis (CE.
434 As can be seen in Fig 3a, several studies use GC-MS for the analysis of opioids in
435 these types of samples. However, this analysis requires a derivation step of the opium
436 alkaloids to make them volatile, what it has limited its applicability, as this step is
437 complex and laborious. Moreover, it is important to take into account the thermolability
438 of the compounds. For this reason, in general, articles using GC are the oldest of the
439 studies collected for the analysis of opioids in these samples (Table 3), and different
440 derivation conditions have been applied. For example, morphine was derivatized by
441 Bjerver et al. (1982) with pentafluoropropionic anhydride (PFPA) for 30 min. Morphine
442 and codeine were determined after derivatisation with TFA (trifluoroacetic anhydride) at

60ºC for 20 min (Hayes et al., 1987) and with 4-dimethylamino pyridine in acetic anhydride at 50ºC for 15 to 30 min (Struempler, 1987). Paul, Dreka, Knight and Smith (1996) quantified morphine, codeine, papaverine and thebaine after acetylation by acetic anhydride and pyridine. Morphine, codeine and thebaine were quantified by after derivatisation with BSTFA (N,O-bis(trimethylsilyl)trifluoroacetamide) mixed with 1% TMCS (trimethylsilyl chloride) (Casella et al., 1997; Meadway et al., 1998; Pettitt et al., 1987; Özbunar et al., 2019) or with pyridine (1:1, v/v) (Yamaguchi et al., 2011). Finally, Van Thuyne et al. (2003) analysed morphine after derivatisation with MSTFA (N-trimethylsilyl-N-methyl trifluoroacetamide) at 80ºC for 10 min. On the other hand, Acevska, Stefkov, Petkovska, Kulevanova and Dimitrovska (2012b) optimized a method based on GC coupled to flame ionization detector (FID) and MS (GC-FID/MS) that did not perform a derivation step before the analysis, which allowed to significantly reduce the total analysis time. This method was validated, and low detection limits (LD) were obtained between 0.91 and 1.95 μg mL^{-1} for morphine, codeine, thebaine, papaverine, noscapine and oripavine. In all of these works, the chromatographic conditions that have been used for the analysis were very similar. Columns containing non-polar stationary phases like Silyl-8 (Bjerver et al., 1982), DB-5 (Cassella et al., 1997; Struempler, 1987; Paul et al., 1996), DB-17 (Pettitt et al., 1987), HP5-MS (Acevska et al., 2012b; Meadway et al., 1998; Özbunar et al., 2019), HP-Ultra 2 (Yamaguchi et al., 2011) and HP-Ultra 1 (Van Thuyne et al., 2003) were used, with helium as the carrier gas. In some studies, the separation was performed under isothermal conditions at 220-250ºC (Bjerver et al., 1982; Hayes et al., 1987; Struempler, 1987; Pettitt et al., 1987), but all others works have used different temperature programs. The detector used by most of them was the MS, which can also be either a quadrupole type (Hayes et al., 1987) or an ion trap detector (Cassella

et al., 1997). Electron impact (EI) ionization was the most commonly used ionization method and the mass spectrum was performed in selected ion monitoring (SIM) mode.

Although GC allows obtaining very low LD, the costly step of derivatization and the possible loss of the analytes has made the authors choose, in the most recent studies, alternative analysis techniques, such as HPLC (Fig. 4a). Different detectors have been coupled with HPLC for the analysis of opioids, as the diode-array detector (DAD) (Acevska et al., 2012a, 2012b; Cao et al., 2007; Meos et al., 2017; Yoshimatsu, Kiuchi, Shimomura & Makino, 20015). In these studies, the most commonly used stationary phases were non-polar: C18 (Acevska et al., 2012a, 2012b; Cao et al., 2007; Yoshimatsu et al., 2005) or C8 (Meos et al., 2017). In some works, reverse-phase liquid chromatography (isocratic or gradient elution mode) was used, with a mobile phase composed by mixtures of methanol (MeOH) with aqueous solutions of trifluoroacetic acid (0.1%) or ammonium acetate (0.5%) and triethylamine to adjust the pH to 9.6 (Acevska et al., 2012a, 2012b; Cao et al., 2007). In other works, ion pair chromatography was used, as an effective reversed-phase technique for separation of ionized organic analytes. In this case, an ion pair reagent such us sodium heptanesulphonate is added to the mobile phase (at pH 3.2 – 3.5) composed by a mixture of acetonitrile and water (Meos et al., 2017; Yoshimatsu et al., 2005).

However, apart from DAD, in recent years there has been an increasing improvement in the development of MS, resulting in detectors with higher sensitivity and specificity. Therefore, the most used detector in the literature for this family of compounds is the MS detector, highlighting the MS/MS detector for its high specificity for the selective mass detection. For all these reasons (Table 3), recent studies are performed with (U)HPLC-MS/MS. The most commonly used type of ionisation source is electrospray ionisation in positive mode (ESI^+) and the most popular MS detector is the triple quadrupole (QqQ).

Almost all of them use multiple reaction monitoring (MRM) which provides even more reliable quantitative data of the analytes (Guo et al., 2013; López et al., 2018; Powers et al., 2017; Sproll et al., 2006; Stranska et al., 2013). In these works, the stationary phase was C18 (Guo et al., 2013; López et al., 2018; Powers et al., 2017; Sproll et al., 2006; Stranska et al., 2013; Zenai et al., 2012). Gradient elution was applied with a mobile phase that consisted in a mixture of MeOH:water (Guo et al., 2013) or MeOH/acetonitrile:water with a low percent of formic acid (Powers et al., 2017; Stranska et al., 2013). In some cases, ammonium carbonate was added to reach pH 9 (López et al., 2018; Sproll et al., 2006). On the other hand, in order to completely minimize the possibility of false positives in real samples, HPLC coupled to triple quadrupole-linear ion trap-tandem mass spectrometry (HPLC-Q_qQ_{LIT}-MS/MS) have been used recently by Xu et al. (2019). The precursors and product ions of the opiates were monitored by MRM and enhanced ion product (EIP) mode. A hydrophilic interaction column (HILIC) was used with ammonium formate:acetonitrile as mobile phase. The analytical column was set a 30º C and the separation of five opiates was carried out in 7 min.

In combination with chromatography, some authors have carried out studies with other techniques, such as CE-DAD and LDI-MS/MS (laser desorption ionization mass spectrometry), which were compared with HPLC. Thus, Meos et al. (2017) proposed CE-DAD as a strong alternative to HPLC-DAD for the analysis of this family of compounds, above all for its simplicity of sample preparation. However, they argued that this was true only at moderate concentrations, and for very low concentrations HPLC is needed because of its higher sensitivity. Furthermore, Skopikova, Hashimoto, Richomme and Schinkovitz (2020) determined opium alkaloids in crude extracts of *P. somniferum* with LDI-MS/MS and later, they compared to classical HPLC-MS/MS. They demonstrated that although HPLC-MS/MS analysis should be performed for precise quantification,

517 rapid qualitative analysis of large sample batches was established, which is very
518 interesting for industrial application.
519
520 *5.3.2 Sample preparation*
521 Nowadays, a high number of sample preparation techniques have been developed,
522 and in most works, the target analytes not only are extracted with organic solvents, so
523 purification techniques that allow, once the extraction stage is done, to purify the extract
524 to eliminate possible matrix interferences in the analysis are also applied. However, as
525 can be seen in Fig. 4b, until now, sample preparation for poppy seeds and food products
526 analysis have been carried out mainly by traditional procedures, such as solid-liquid
527 extraction (SLE). Many types of organic solvents have been evaluated for SLE, but the
528 most widely used is MeOH, either alone or with a low percentage of acid (Table 3). An
529 example is a study of Sproll et al. (2006) that developed a method to analyse morphine
530 and codeine in poppy seeds by HPLC-MS/MS. In this work, after optimization of the
531 extraction parameters, 10 g of the seeds were extracted with 30 mL of MeOH with 0.1%
532 acetic acid during 60 min in an automatic shaker at 250 rpm. The precision resulted in
533 ranges between 7.4 and 9% (relative standard deviation, RSD) and the accuracy was
534 between 9.8 and 17.6% (relative error). In the same way, Acevska et al. (2012a, 2012b)
535 used only MeOH for the extraction of morphine, codeine, thebaine, papaverine, noscapine
536 and oripavine in poppy straw. In these works, 5 mL of extraction solvent was added to
537 0.1 g of sample, the mixture was sonicated for 20 min (40ºC) and then, it was centrifuged
538 at 4000 rpm for 5 min. The extraction was performed twice, and the supernatants were
539 mixed for further analysis by HPLC-DAD or GC-FID/MS. Good recovery values were
540 obtained in both studies, near to 100%, with low RSD. Similarly, in a recent study of

Skopikova et al. (2020), 0.04 g of the powdered plant was extracted with 1.55 mL of MeOH and the crude extracts were analysed by LDI-MS/MS for a rapid opiate detection.

Looking at these good recovery values, it seems that MeOH is a good extraction solvent for SLE. However, other extraction solvents have been used. For example, Cao, Li, He, Li & Liu (2007) optimised an extraction method based on aqueous two-phase system (ATP) containing 6% PEG (poly(ethylene glycol) with a molecular mass of 4000 and 30% $(NH_4)_2SO_4$ to analyse papaverine in pericarpium papaveris by HPLC-DAD. Results were compared with Soxhlet extraction, with 40 mL of MeOH during 4 h. Adequate recovery values (97.3%, RSD $\leq$ 1.8%) were achieved, with the advantage that the developed method is more environmentally benign and cost effective because required less time. These good results could be based on hydrophobic interaction between papaverine and ATPS system. In a more recent study, López et al. (2018) evaluated the effectiveness of different types of organic solvents for the analysis of opium alkaloids in poppy seeds and bakery products by UHPLC-MS/MS. A mixture of acetonitrile:water:formic acid (80:19:1, v/v/v) was selected for this purpose because it was more effective on ground poppy seeds. 10 g of the samples were mixed with 100 mL of the solvent for 30 min. Recovery values in the range of 70 - 120%, (RSD $\leq$ 20%), were obtained except for noscapine (150 - 170%). The presence of interferences from the matrix may be the reason for the non-satisfactory results in the case of noscapine. Finally, in other works, after alkalinisation to pH 9 – 9.5 (with carbonate or ammonium buffers), the opiates were extracted from poppy seeds with mixtures of chloroform:isopropanol, dichloromethane:isobutanol or dichloromethane:MeOH (90:10, v/v). Usually, to reduce interferences, the organic extract is then acidified (with sulphuric or hydrochloric acid), after this the pH of the aqueous phase is adjusted again to pH 9 - 9.5 and, finally, a re-extraction with the same mixture of solvents is carried out. This complex sample

preparation protocol was coupled, in all cases, with analysis by GS-MS after derivatization of the opium alkaloids (Meadway et al., 1998; Paul et al., 1996; Pettit et al., 1987; Struempler, 1987; Yamaguchi et al., 2011).

For liquid samples (teas), liquid-liquid extraction (LLE) was applied by Van Thuyne et al. (2003) using methanol:dichloromethane, after adjusting the pH to 9.5, to analyse morphine by GC-MS. More recently, in homemade poppy seed teas, obtained under different conditions to simulate home brewing, Powers et al. (2017) quantified morphine, codeine and thebaine. In this work, any sample treatment was applied, and the tea was directly analysed by HPLC-MS/MS after appropriate dilutions. Overall accuracy ranged from 92.3 – 103.4% with minimal matrix effect.

As can be seen, in all the previously discussed studies only organic solvents were used for the extraction of the target analytes. However, to obtain a good clean extract avoiding the interference of the matrix components, and thus achieving good recovery values, a further purification step by solid-phase extraction (SPE), with different types of sorbents, is included in some other works (Table 3). For example, in 1982, Bjerver et al. used 15 g of diatomaceous earth to purify the aqueous extract obtained from blue and white poppy seed (5 g of sample and 10 mL of 0.1 M citric acid, pH 4). More recently, Meos et al. (2017) determined morphine, codeine and papaverine in dry poppy capsules of 34 different cultivars used as ornamental plants. 0.5 g were mixed in ultrasound with 20 mL of ethanol:water (50:50, v/v). After dilution and adjustment to a pH of 9.5, 1 mL was passed through the SPE column (8 cm x 10 mm, filled with diatomaceous earth) and the analytes were eluted with 2-propanol:dichloromethane and analysed by HPLC-DAD. In the same way, Hayes et al. (1987) determined morphine and codeine in black poppy seeds from the USA. Samples (1g) were homogenized in 5 mL of 0.1 M citrate buffer and then passed through a Chem Elute column, which was filled with diatomaceous earth. After

591 washing with water, the analytes were eluted with chloroform and analysed by GC-MS.
592 In other works, Clean Screen® DAU extraction column (200 mg) have been used
593 (Cassella et al., 1997; Özbunar et al., 2019). In both studies, firstly a solvent extraction
594 with 5 - 10 mL of MeOH was carried out (0.2 and 0.5 g poppy seeds), the extracts were
595 loaded in conditioned cartridges and analytes were eluted with ethyl
596 acetate:isopropanol:ammonium hydroxide (84:12:4, v/v/v). After evaporation and
597 derivatization, the analysis was carried out by GC-MS. Finally, in other works Oasis®
598 MCX have been selected for the purification stage (Guo et al., 2013; Stranska et al., 2013;
599 Yoshimatsu, et al., 2005), and the comparison with other cartridges such as Oasis® HLB
600 was carried out. For example, Yoshimatsu et al. (2005) analysed morphine, codeine,
601 thebaine, papaverine and noscapine by HPLC-DAD in powdered poppy capsules. For this
602 purpose, 0.05 g of the sample was mixed with 5 mL of the extraction solvent (water, 5%
603 acetic acid, 0.1 M sodium citrate buffer, pH 6.0) under sonication. An aliquot of the
604 supernatant was purified by SPE (either Oasis® HLB or Oasis® MCX) and the influence
605 of the extraction conditions was evaluated. Results obtained indicated that substances that
606 affected morphine separation could not be removed with Oasis® HLB. For this reason,
607 finally the Oasis® MCX cartridge was used and get good recoveries between 99.94 and
608 112.18% (RSD $\leq$ 1.35%). In a similar way, Guo et al. (2013) evaluated the use of both
609 types of cartridges to analyse morphine, codeine, papaverine, noscapine and thebaine in
610 hot pot broth by HPLC-MS/MS. First, 20 mL of HCl was mixed with 5 g of hot pot and
611 an ultrasonic extraction was carried out. After, to remove fats, a second extraction with
612 petroleum ether was applied. Once the water phase-extracts were purified by SPE, good
613 recovery values were obtained with both cartridges, except for morphine using Oasis®
614 HLB (only a 10% recovery). Moreover, with Oasis® MCX better purification results (72
615 - 124%, RSD $\leq$ 25%) were obtained, because of its high specificity for basic compounds.

One of the main drawbacks of SPE is the considerable consumption of organic solvents that presents problems with waste generation. Therefore, a current objective of researchers is to develop new sample preparation techniques that require much fewer amounts of organic solvents and are therefore not only more environmentally friendly but also simpler and faster. In addition to this, many research studies have focused on the development of nano-sized sorbent materials for the pre-concentration of target analytes and to reduce matrix effects. One example is the work of Xu et al. (2019) who synthesized a new sorbent with the aim of determining possible opium alkaloid amounts in different Chinese hot pot samples. This synthesis was based on the formation of magnetic Fe_3O_4 particles, coated with non-porous silica, functionalized with amantadine (Fe_3O_4@SiO_2@ADME material). Once the material was synthesized, the sample was extracted and purified by magnetic solid-phase extraction MSPE. Firstly, 2 g of sample (diluted with water) was extracted with acetonitrile. The supernatant was evaporated, and water was added for the subsequent purification of this mixture by MSPE under optimized conditions. Good recovery values, between 80 and 115% (RSD 4.3 - 10.7%) for morphine, codeine, thebaine, papaverine and noscapine were observed. Therefore, the results obtained in this work demonstrated the advantages of MSPE compared to SPE, as fewer volumes of organic solvent and amount of sorbent (50 mg) was used.

6 Future perspectives and conclusions

Several actions that can be taken to control the levels of opium alkaloids in food products, as establishing: a) the maximum limits in seeds or food, b) a classification of different varieties of poppy plants with seeds exclusively for food, c) good harvesting practices to minimize contamination and d) good processing practices to minimize the

concentration of opium alkaloids. Nowadays there are very few studies of opium alkaloids in food samples, there are mainly in biological samples. In addition, as can be seen in Fig. 5 studies of opium alkaloids are mainly about morphine, and the other alkaloids have been less studied, especially, noscapine, narceine and oripavine. Thus, in order to know the real exposure of consumers and to be able to make a characterization of the hazard of its intake, there is a need to carry out more studies on the quantities of all opioids in poppy seeds and foods with poppy seeds available on the market.

To perform these studies, researchers must consider that the quantities of these compounds are very low and are found in very complex matrices. In this sense, it is essential to develop and validate new analytical methods based on appropriate sample preparations and selective analytical techniques. Most literature reports use HPLC-MS/MS and conventional extraction techniques, such as solvent extraction and SPE. However, it should be emphasized that, nowadays, sample preparation techniques are evolving towards more sophisticated and environmentally friendly modes, for example by the application of new materials (Casado, Pérez-Quintanilla, Morante-Zarcero, & Sierra, 2017; Sierra & Morante-Zarcero, 2018). In addition, great advances have been made in the food sample preparation field, involving the development of new materials and their integration in micro-extraction techniques to determine natural toxins. Among all, mycotoxins have been by far the most studied over the last years (Casado et al., 2020). Nevertheless, the development of advanced analytical strategies for the determination of other natural toxins such as opium alkaloids in seeds or foods, through the combination of new materials and micro-extraction techniques, is still a great future challenge.

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669 **References**

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824 **Figure Captions**

825 **Fig. 1.** Diagram showing the aspects to be included in this review.

826 **Fig. 2.** Number of RASFF (Rapid Alert System for Food and Feed) notifications of
827 morphine and morphine alkaloids per year from 2005 to 2020.

828 **Fig. 3.** Different (a) analytical techniques and (b) sample preparation treatment used for
829 the determination of opium alkaloids in poppy (seeds and plant), foods with poppy seeds
830 and poppy teas (from 1982 until 2020). For details, see Table 3.

831 **Fig. 4.** Opium alkaloids that have studied in poppy seeds or poppy seed foods in articles
832 published between 1982 and 2020.

833

Figure

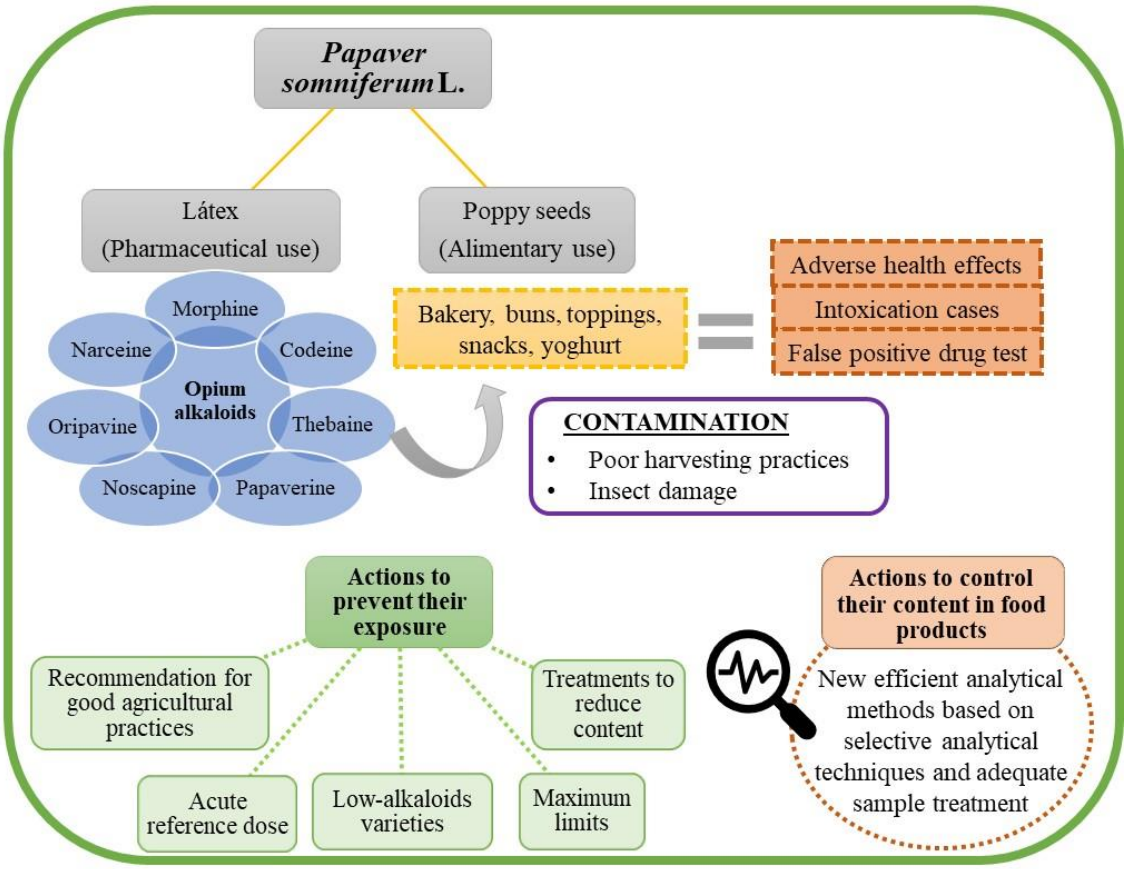
Papaver somniferum L.
Látex (Pharmaceutical use)
Poppy seeds (Alimentary use)
Morphine
Narceine
Codeine
Opium alkaloids
Oripavine
Thebaine
Noscapine
Papaverine
Bakery, buns, toppings, snacks, yoghurt
Adverse health effects
Intoxication cases
False positive drug test
CONTAMINATION
Poor harvesting practices
Insect damage
Actions to prevent their exposure
Recommendation for good agricultural practices
Treatments to reduce content
Acute reference dose
Low-alkaloids varieties
Maximum limits
Actions to control their content in food products
New efficient analytical methods based on selective analytical techniques and adequate sample treatment

Fig. 1.

Figure

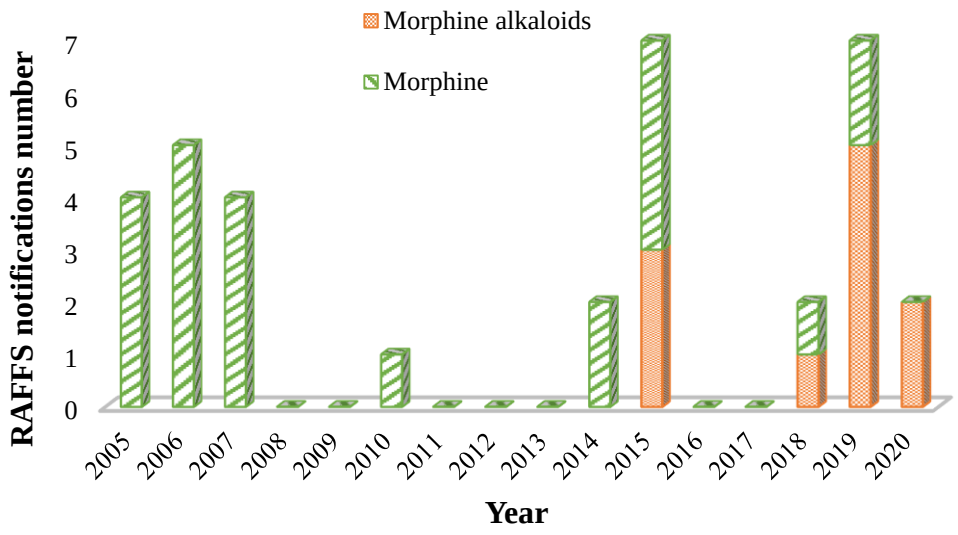
Morphine alkaloids
Morphine
RAFFS notifications number
0
1
2
3
4
5
6
7
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016
2017
2018
2019
2020
Year

Fig. 2.

Figure

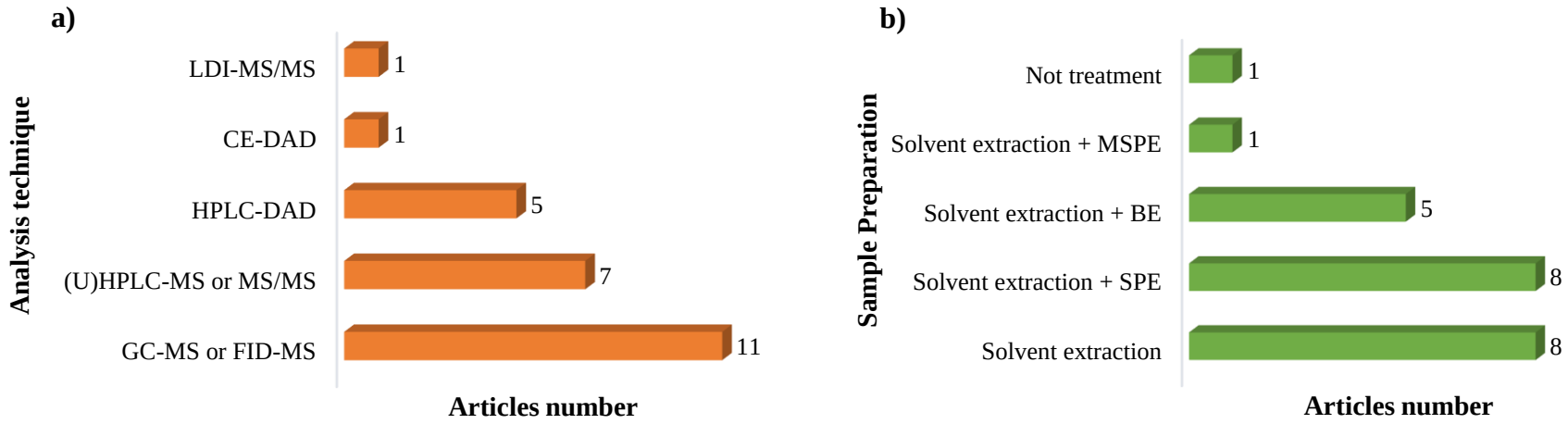
a)
Analysis technique
LDI-MS/MS
CE-DAD
HPLC-DAD
(U)HPLC-MS or MS/MS
GC-MS or FID-MS
1
1
5
7
11
Articles number
b)
Sample Preparation
Not treatment
Solvent extraction + MSPE
Solvent extraction + BE
Solvent extraction + SPE
Solvent extraction
1
1
5
8
8
Articles number

Fig. 3.

Figure

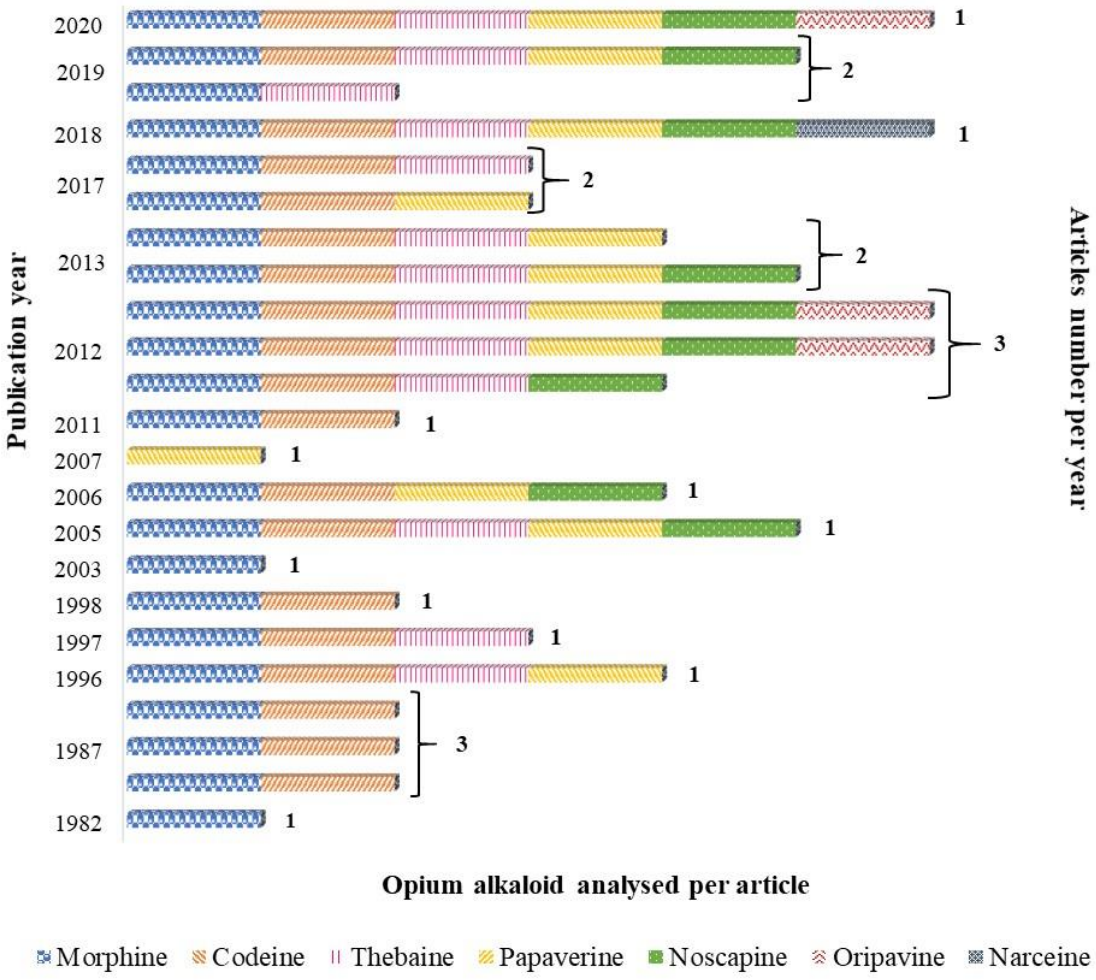
Publication year
2020
2019
2018
2017
2013
2012
2011
2007
2006
2005
2003
1998
1997
1996
1987
1982
1
2
1
2
2
3
1
1
1
1
1
1
1
1
3
1
Articles number per year
Opium alkaloid analysed per article
Morphine
Codeine
Thebaine
Papaverine
Noscapine
Oripavine
Narceine

Fig. 4

Table

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