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Investigating the effect of different grinding conditions and methods on the concentration of opium alkaloids in poppy seeds as a good reduction practice

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ABSTRACT

In recent years, following intoxications and false positive drug tests for poppy seeds, dangerously high concentrations of opium alkaloids (OAs) have been found. For this reason, health authorities are demanding studies to establish effective practices to reduce OAs in these seeds. Grinding, a common culinary process for various recipes, has previously been associated with potential morphine degradation. However, these studies have not established effective grinding conditions to decrease the concentration of all main OAs. Furthermore, due to the large variation in OAs concentration that can occur even in the same bag, this can be a difficult task. In view of the above, this study aimed to evaluate for the first time the impact of different grinding types, varying grinding time or repetitions, to determine any degradation trends of the main OAs present in poppy seeds. As a result, it was obtained a notable 20% degradation of morphine in the second grinding with grinders where the oil content of the seeds is not released, hinting at oil's protective role against morphine oxidation. Therefore, the choice of grinding method is crucial. However, while acknowledging this, relying solely on this method as a good practice for OAs reduction may be insufficient, as it seems to have minimal impact on other OAs with any of the tested methods and conditions.

1. Introduction

There is a trend to incorporate various types of seeds into food due to their beneficial nutritional properties, such as poppy seeds derived from the *Papaver somniferum* L. plant (Ghafoor et al., 2019; Musa Özcan and Atalay, 2006). These seeds have a traditional history of use in many Central European countries and are now gaining popularity in other regions (AESAN (Spanish Food Safety and Nutrition Agency), 2020; EFSA (European Food Safety Authority), 2018). Poppy seeds can serve as toppings for salads, yoghurts, and in various bakery products such as bread, biscuits, and buns. Furthermore, they can be used to create soothing infusions (Powers et al., 2018; Li et al., 2021). However, it is quite common to use ground poppy seeds, incorporating them into salads or as fillings in traditional sweet dishes and cakes.

The concern with using these seeds is their potential contamination with opium alkaloids (OAs) (Meos et al., 2017; Montgomery et al., 2020; Özbunar et al., 2019; Yamaguchi et al., 2011). This phenomenon could be linked to automated harvesting methods that result in the seeds coated with the plant's latex, containing elevated levels of OAs (Casado-Hidalgo et al., 2021a). The consumption of food contaminated with high concentrations of OAs has led to severe cases of intoxication and, in some instances, even false positive drug tests results (Meadway et al., 1998; Newmeyer et al., 2015; Rohrig & Moore, 2003; Van Thuyne et al., 2003). The main five OAs can be found in these seeds are morphine, codeine, thebaine, papaverine and noscapine (EFSA, 2018). However, currently, only morphine and codeine have been considered. Present legislation addresses these two toxins exclusively, setting a limit for seeds at 20 mg/kg of morphine equivalent (calculated as morphine + 0.2 × codeine) (Commission Regulation (EU), 2023). Nevertheless, previous studies have shown that seeds may contain notably high concentrations of other OAs, which health authorities suggest could potentially be even more toxic (BfR, (German Federal Institute for Risk Assessment), 2006; EFSA, 2018; Eisenreich et al., 2020). Hence, it is vital not just to test for morphine and codeine in these samples to ensure compliance with

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regulations, but also to analyse the other OAs present. This step is crucial for accurately assessing consumer exposure and subsequently establishing appropriate legislation.

To ensure that poppy seeds comply with legislation and are safe for consumers, health authorities are calling for good practices to prevent and reduce the presence of OAs in food (European Commission, 2014; Kaltner, 2022). Therefore, studies have been undertaken to assess the impact of thermal processing on bakery products (Carlin et al., 2020; Shetge et al., 2020; Shetge & Redan, 2022; Vera-Baquero et al., 2022) and lactic acid fermentation in yoghurt (Casado-Hidalgo et al., 2023b), demonstrating that high temperatures and fermentation can degrade OAs in poppy seeds. However, although there are some studies that ensure morphine reduction with grinding (Avula et al., 2023; European Commission, 2014; Sproll et al., 2006, 2007), none of them have established specific grinding conditions, and it is unknown whether the type of grinding or other variables, such as grinding time, can significantly influence morphine concentration in seeds. In addition, the impact of grinding on all the main OAs that might be present in seeds has not been studied (Casado et al., 2023). Therefore, it is very important to evaluate which conditions of this widespread culinary practice with poppy seeds can ensure a decrease of OAs and thus ensure food safety.

Hence, the aim of the present work is to evaluate the potential impact of grinding on the concentration of OAs in poppy seeds. To achieve this, an evaluation of five different grinding methods, some of them with a more industrial application and others more for domestic use, was carried out to assess all possible approaches and modes of grinding poppy seeds and to determine whether there are differences between different types of grinders with different grinding times or consecutive grindings. To conduct this research, an analytical methodology previously optimised and validated by our research group was utilized to quantify OAs in whole poppy seed and another validated method developed to analyze OAs concentration in each grinding study of ground poppy seeds.

2. Materials and methods

2.1. Reagents

HPLC-MS grade acetonitrile (CAS 75–05–8) and methanol (CAS 67–56–1) were obtained from Scharlab (Barcelona, Spain). LC-MS grade formic acid 99% Optima™ (CAS 64–18–6) was purchased from Fisher Chemical (Madrid, Spain). Ammonia 32% (w/w) (CAS 1336–21–6) and hydrochloric acid 36% (w/w) (CAS 7647–01–0) were acquired from Scharlab (Barcelona, Spain). Ultrapure water (resistivity 18.2 MΩ cm) was acquired from a Milli-Q water purification system (Millipore, Billerica, MA, USA).

For the reagents and materials required to carry out the synthesis of each of the adsorbent materials used in the purification step, see Supplementary Information 1.

2.2. Standards and solution preparation

Standards of morphine, codeine and thebaine were purchased from Alcaliber S.A.U. (Madrid, Spain). Noscapine, papaverine, morphine-d_3 and codeine-d_3 (internal standards, IS) were acquired from Sigma-Aldrich (Zwijndrecht, The Netherlands).

Individual stock solutions were prepared in methanol with concentrations of 1000 μg/mL of each of the OAs. The working standard solutions were prepared by serial dilution of stock solutions with methanol to 1 μg/mL. The internal standard was spiked from a standard of 1 μg/mL of each of them (morphine-d_3 and codeine-d_3). All previous solutions were stored in light-protected at −20 ℃.

2.3. Samples

A sample of poppy seeds was acquired from a supermarket in Madrid, Spain, specifically for this study. The packaging indicated their origin as Turkey and included usage recommendations, suggesting incorporation into juices, soups, smoothies, and yoghurts, as well as mentioning the feasibility of grinding them.

2.4. Analysis methodology for quantification of opium alkaloids in whole and ground poppy seeds

To compare the concentrations obtained from both whole and ground poppy seeds, optimised and properly validated methods from our previous studies for each sample were employed. To analyse OAs in the sample of whole poppy seeds, the method of Casado-Hidalgo et al., 2021a was used. This method was based on a solid liquid extraction (SLE), a purification by magnetic solid phase extraction (MSPE) followed by HPLC-MS/MS analysis. First, to do the SLE, 2.5 g of poppy seeds were extracted with a double extraction with 30 mL of MeOH/water, 50/50 (v/v). This mixture was vortexed for 30 s (Rx^3 Velp Scientifica, Usmate, MB, Italy) and stirred magnetically for 30 min. The two supernatants were mixed, and 2 mL were purified by MSPE. For this purpose, 50 mg of $Fe_3O_4@SiO_2@mSiO_2$ material was added to the extract and subjected to 1 min of ultrasound. Then, with an external magnet, the solution was decanted, and the analytes were eluted with a double desorption with 2 mL of diethyl ether/MeOH 80/20, v/v by 1 min of ultrasound. Finally, 2 mL of this supernatant was evaporated under vacuum and reconstituted in 950 μL of water/AcN 90/10, v/v with 1% formic acid and 50 μL of a 1 μg/mL dilution of morphine-d_3 and codeine-d_3 (IS) to analysed by HPLC-MS/MS.

On the other hand, to analyse ground poppy seeds, the protocol by Casado-Hidalgo et al., 2023a was used. In this case, the extraction was carried out by ultrasound assisted extraction (UAE), a purification by solid phase extraction (SPE) followed by HPLC-MS/MS analysis. First, to do the UAE, 0.5 g of ground poppy seeds were extracted with 8.5 mL of MeOH with 1% HCl. The mixture was vortexed for 10 s and sonicated for 5 min and 48 s (Sonopuls HD 3100, Bandelin, Berlin, Germany) with an MS 73 probe with a diameter of 13 mm at 75% amplitude in pulse mode. Subsequently, the mixture was centrifuged at 9000 rpm for 5 min to recover the supernatant (Digicen 21 R from Ortoalresa, Madrid, Spain). Then, the extract was purified by SPE with a silica SBA-15 functionalised with sulfonic groups (SBA-15-SO_3^-) material. In addition, to make the cartridges, polyethylene frits (0.20 μm) and nylon filter membranes (0.45 μm) from Scharlab (Barcelona, Spain) were used with 25 mg of SBA-15-SO_3^- material. First, a conditioning step was performed with 2 mL of water with 1% HCl, a loading with 2 mL of the extract diluted 1/10 with water with 1% HCl and adjusting the pH to 1 and an elution with 2 mL of MeOH with 1% ammonia. Finally, an aliquot of 950 μL was taken, and 50 μL of a 1 μg/mL dilution of morphine-d_3 and codeine-d_3 (IS) was added prior to HPLC-MS/MS analysis.

The analysis of OAs was carried out following our previous works (Casado-Hidalgo et al., 2021a; Casado-Hidalgo et al., 2023a). For this purpose, a Varian 1200/1200 LC (Varian Ibérica, Madrid, Spain) coupled to a triple quadrupole tandem mass spectrometer detector (1200 L TQ) with electrospray ionisation (ESI) ion source was used. The data acquisition system used was MS Workstation Varian (version 6.8). The autosampler used was a ProStar 410 with a 100 μL loop. The column used was a C18 Kromaphase 100 (150 × 2.0 mm, particle size 3.5 μm, Scharlab, Barcelona, Spain) at 30 ℃. The injection volume was 10 μL (partial injection) with a gradient elution of water (A) and acetonitrile (B), both with 0.1% formic acid, as follows: 90–30% A (0–6 min), 30–90% A (6–9 min) and 90% A (9–11 min) to re-equilibrate the column. The flow rate was set at 0.25 mL/min. On the other hand, mass spectrometric acquisition was by electrospray ionisation in positive mode (ESI+) with multiple reaction mode (MRM). The drying gas was N_2 at 350 C and 22 psi, and the nebuliser N_2 at 58 psi. The capillary voltage was 5000 V, and the shielding was 600 V. The collision gas was argon at 1.90 mTorr, and the detector voltage was 1480 V. The mass peak width of Q1 2.5, mass peak width of Q3 2.5 and the MRM scan width was 0.5 s. The cone voltage was 72 V. Table S1 shows the optimal parameters for the analysis with their retention time, precursor ion, transitions, ionization mode and collision energy of each compound.

2.5. Different types and conditions of grinding evaluated

To assess the potential degradation impact of OAs after grinding of poppy seeds, an evaluation was undertaken to determine the presence of any degradation patterns concerning either the grinding time or successive grindings. Five different types of grinding were evaluated to see if there are differences between the different types of grinding on the effect of OAs concentration in poppy seeds. Additionally, varying handling conditions were assessed for each, considering their respective operating capacities. This involved evaluating different parameters such as grinding times (1, 2, 5 and 7 min) or, alternatively, the number of consecutive grindings (up to 3 times) whenever applicable. To achieve this, the sample was introduced initially, and subsequently, at various time intervals or consecutive grindings, three sample replicates were collected for analysis.

2.5.1. Grinding experiment type 1

In this experiment a Mortar Grinder (RM200, Retsch, Haan, Germany), that could be used on an industrial scale, was used. The mortar has a pestle that can crush and mill the material in a mortar containing the sample to be milling while a scraper mixes the milled material and scrapes off material adhering to the mortar. In this case, grinding was evaluated with different grinding times studied with each of these: 1, 2, 5, 7, 10, 30 and 60 min.

2.5.2. Grinding experiment type 2

An Ultra Centrifugal (Mill ZM 100, Retsch, Haan, Germany), that could be used on an industrial scale, was used. Grinding takes place in the ultra-centrifugal mill by the impact and shearing action between the rotor and the fixed ring sieve. The sample passes through the funnel onto the rotor. With the centrifugal acceleration, it is hurled outwards with great energy and is pre-crushed on the wedge-shaped rotor teeth before being finely ground between the rotor and the screen. In this case, the grinding times evaluated were 1, 2, 5 and 7 min.

2.5.3. Grinding experiment type 3

A Mincer (A11 Basic analytical mill, IKA, Staufen, Germany) with impact milling, which could be employed for home use, was used. This grinder has a beater rotor, and thus, the grinding material is broken up. The granularity of the final product is determined by the duration of the grinding. In this case, the grinding times also were 1, 2, 5 and 7 min.

2.5.4. Grinding experiment type 4

A Poppy Seed Mill (Poppy Seed Mill, Westmark, Elspe, Germany), that could be employed for home use, was used. This mill has a hand crank for manual turning, which causes a ceramic worm to rotate and pass the sample through a pinion disc, where it is crushed by friction. In this case, 10 g of sample were taken, and as the grinding time could not be controlled in this device, the alternative that was carried out to see a possible increase in degradation was to grind consecutively the initial 10 g of sample. Therefore, it was evaluated with 1, 2 and 3 grindings.

2.5.5. Grinding experiment type 5

A Grinding Coffee (Grinding Coffee, Day Day Fun, Hong Kong, China), that could be employed for home use, was used. To do the assays, 10 g of sample were evaluated by grinding once and twice.

2.6. Statistical analysis

Statistical analysis was performed using SPSS 25.0 statistical package (SPSS INC., Chicago, Il, USA) by analysis of variance (ANOVA) using Duncan's multiple range test. Significant differences were considered significant for p values ≤ 0.05. In the case of the test where only two levels of a single factor were assessed, a student's t-test with two tails and a 95% confidence interval was performed. In both cases, significant differences were considered significant for p values ≤ 0.05 and different letters were used to indicate this.

3. Results and discussion

3.1. Evaluation of the effect of grinding method on the concentration of opium alkaloids in poppy seeds

Assessing the effect of a culinary processing type on opium alkaloids can be a difficult task. This has been demonstrated by the contradictory results obtained for other types of processing such as heat treatment, with some authors claiming a considerable degradation effect (Vera-Baquero et al., 2022) and others that there is no significant effect (Shetge et al., 2020). For this reason, the consensus emphasizes the need for meticulous study design, considering and accounting for all variables involved (Fleischman et al., 2021; Kuntz et al., 2021). So, in this study, two key issues were considered, specifically the large variability of opium alkaloids in the seeds, and the analytical methodology used and validated for the type of samples involved.

The large variability of opium alkaloids presents in the seeds, even among seeds obtained from the same bag has been confirmed in numerous studies (Carlin et al., 2020; Casado-Hidalgo et al., 2021a; López et al., 2018). This variability may be result from external contamination and is influenced by factors such as plant variety, climate, harvest timing and, and notably, the harvesting method. Presently, automated harvesting methods contribute to seed impregnation with latex, exacerbating this variability (EFSA, 2018; Meos et al., 2017; Stranska et al., 2013). Consequently, obtaining an initial value of OAs in the seeds for studying the influence of processing, especially grinding, becomes challenging due to this variability. This problem is not as acute when the seeds are ground, as the concentration of OAs in that portion of seeds is homogenised. For this reason, it is important to carry out numerous replicates of whole seeds to obtain a mean value with as low a standard deviation as possible. In consequence, twelve replicates of the sample were analysed with the validated method by Casado-Hidalgo et al. 2021a, and the concentration ranges obtained were for morphine from 2.5 to 8.8 mg/kg, for codeine from 0.5 to 2.2 mg/kg, for thebaine from 0.1 to 1.5 mg/kg, for papaverine from 0.5 to 2.8 mg/kg and noscapine from 0.2 to 4.5 mg/kg. These results were according to previous works that large variations of OAs were obtained even in the same bag, and for this reason, they gave the concentration of OAs in ranges (Carlin et al., 2020; Casado-Hidalgo et al., 2021a; López et al., 2018). For all of this, comparing OAs concentrations in whole and ground poppy seeds can be a complicated task, and it is easy to make wrong conclusions. Therefore, in the present work, it also has been decided to evaluate whether there is a trend of degradation of OAs in the seeds with increasing grinding time or with consecutive grindings (Sections 3.1.1 and 3.1.2). In this way, it can be determined whether this type of culinary processing is effective in eliminating or preventing the presence of OAs in seeds, as stated by EFSA in their recommendation of 2014 (European Commission, 2014).

Furthermore, it is important to note that when grinding the seeds, the matrix becomes different and more complex compared to whole poppy seeds. This complexity could stem from the release of fatty acids on the seed's surface, forming a fatty paste. It is crucial to consider this aspect, as inadequate analyte recovery or a significant matrix effect could lead to inaccurate results and consequently wrong conclusions. For this reason, in the present work, another previously developed analytical methodology was used to effectively analyse ground seeds (Casado-Hidalgo et al., 2023a). The analysis method was previously validated in terms of linearity, limits of detection and quantification of the method, inter- and intra-day precision and accuracy at three concentration levels, and the matrix effect and selectivity were also evaluated

Table 1
Matrix-matched calibration curves corrected with isotope-labelled IS employed to quantification of OAs in ground poppy seeds.

Analytes	Matrix-matched calibration curves		Accuracy[b]	Precision[c]	
	Equation	RSD (%)[a]	(Recovery, % ± SD)	Intra-Day Precision (RSD %)	Inter-Day Precision (RSD %)
Morphine	8.79x + 0.64	1	98 ± 8	4	8
Codeine	21.60x – 0.01	4	98 ± 2	1	2
Thebaine	54.95x + 3.02	6	93 ± 4	3	5
Papaverine	170.44x + 10.12	2	100 ± 5	3	5
Noscapine	239.66x + 41.98	1	101 ± 5	3	5

[a] RSD (%): relative standard deviation between five replicates of matrix-matched calibration lines adjusted with IS. [b] Accuracy and [c] precision were obtained by spiking sample at 3.4 mg/kg (similar concentration found in the sample).

(Casado-Hidalgo et al., 2023a). As all the results were successful and no matrix effect was present, this methodology can be applied for the quantification of OAs in poppy seeds. However, to ensure the correct quantification of all ground seed studies and all replicates of each of them, in the present work, matrix-matched calibration curves corrected with IS were used to quantify and correct for the possible matrix effect in the samples analysed, which are shown in Table 1. Besides, to ensure their reproducibility, the relative standard deviation (RSD %) was calculated between the slopes of the calibration lines used, obtaining in all cases a result of less than 6% (Table 1). These matrix-matched calibration curves were prepared with a blank poppy seed matrix free of OAs. For this purpose, sample extracts obtained after the same analytical procedure were spiked with an aliquot of a standard solution containing the OAs according to the required concentration level of the calibration curve. In addition, an isotope-labelled IS correction was performed by adding 50 μL of 0.1 μL/mL of each IS to each point of the matrix-adjusted calibration curves (Table 1). In addition, to confirm the viability of the previously validated method, precision and accuracy were determined at 3.4 mg/kg, a concentration like that found in the sample analysed in this work. To calculate it, the areas obtained for simulated samples (blank samples spiked with this concentration but before HPLC-MS/MS analysis) were used. To accuracy, recovery values were obtained between 93% and 101% (Table 1). Furthermore, to evaluate the precision, repeatability (intra-day precision, RSD %) was calculated six times on the same day and reproducibility (inter-day precision, RSD %) was estimated with three replicates on three different days, obtained values lower than 4 and 8%, respectively (Table 1).

Therefore, as shown in Fig. 1, five different types of grinding were studied, and three of the grinding types used in the present work had the possibility to select the grinding time (Section 3.1.1). On the other hand, two other grinder types consisted of a hand crank that rotates a ceramic worm and passes the sample through a pinion disc where it is crushed by friction. Thus, in this case, instead of evaluating different types of grinding or consecutive grindings were evaluated (Section 3.1.2). Subsequently, these seeds were subjected to each of the types of grinding and the specific conditions evaluated, and once ground, three replicates were taken and subjected to the proposed analysis methodology. Once the concentrations of the ground seeds were obtained with each of the conditions, a comparison was made with the concentration obtained in the whole seeds (is the value determined as time zero or control) to determine if there were significant differences and thus to check if there was a tendency towards degradation with increasing grinding time or

Fig. 1. Types of grindings evaluated with the grinding conditions studied in each of the types (amount of sample and times or consecutive grindings) and the appearance of the ground poppy seed sample.

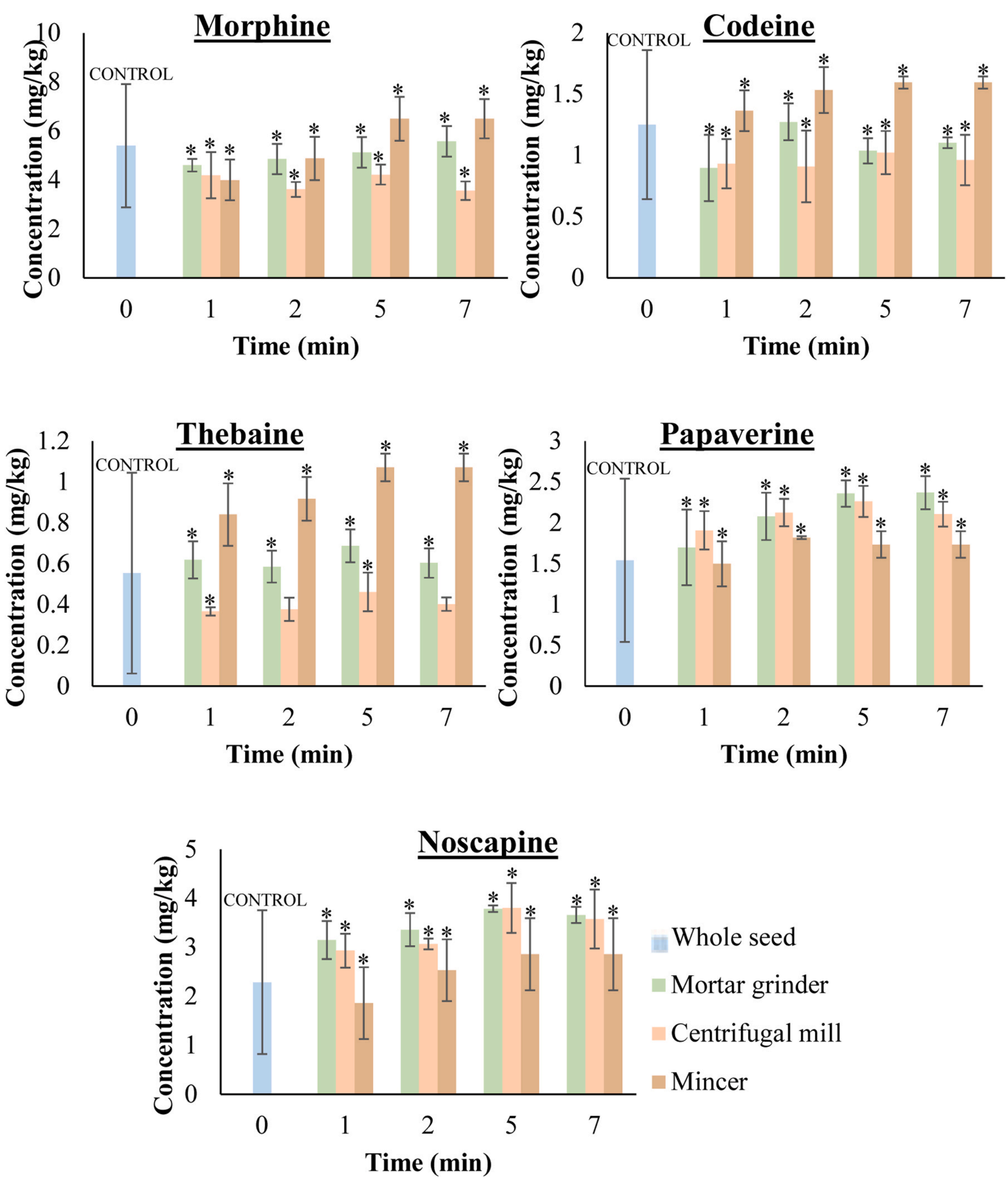

Fig. 2. Concentrations of each of the OAs determined in poppy seeds ground with different types of grinding (mortar grinder, centrifugal mill, and mincer) for different grinding times (1, 2, 5 and 7 min). * Mean that there are no statistically significant differences and ** there are statistically significant differences ($p \leq 0.05$) between the different times with control.

with successive grindings.

3.1.1. Effect of grinding time

Different grinding times were used with grinders where the grinding time could be controlled as explained above (mortar grinder, centrifugal mill and mincer), specifically 1, 2, 5 and 7 min for all of them and also 10, 30 and 60 min in the case of the mortar grinder, which higher time can be controlled.

As shown in Fig. 2, none of the studies performed showed significant differences compared to the control (time 0). Besides, Table 2 shows whether the different grinding times showed statistically significant differences ($p < 0.05$) between them and none of the cases showed statistically significant differences in the concentration of OAs in the different conditions. Thus, it was concluded that there was no trend towards degradation of OAs with increasing grinding time with any of the different types of grinders. This was because no significant differences were shown with the control (time 0) as shown in Fig. 2, neither between the different times evaluated as shown in Table 2. The explanation for this result could be because this type of grinding extracts the oil from the seeds to form a paste. The fact that this type of grinding does not result in a significant opium degradation effect could be due to the protective effect of the oil against oxidation of the OAs.

It should be noted that possible differences were shown between the different studies (experiments 1, 2 and 3). But this could be due to the high dispersion of OAs in poppy seeds, even in the same bag, as seen in previously published papers (Carlin et al., 2020; Casado-Hidalgo et al.,

Table 2
Compilation of all the results obtained in each of the grinding studies which is possible to evaluate the grinding time.

Grinding type	Time (min)	**Morphine**	**Codeine**	**Thebaine**	**Papaverine**	**Noscapine**
Mortar grinder	1	$4.6 \pm 0.3^{a,b}$	0.9 ± 0.3^{a}	0.6 ± 0.1^{a}	$1.7 \pm 0.5^{a,b}$	3.2 ± 0.4^{a}
	2	$4.9 \pm 0.6^{a,b}$	$1.3 \pm 0.1^{a,b}$	0.6 ± 0.1^{a}	$2.1 \pm 0.3^{a,b}$	3.4 ± 0.4^{a}
	5	5.1 ± 0.6^{b}	$1.1 \pm 0.1^{a,b}$	0.7 ± 0.1^{a}	2.4 ± 0.2^{b}	3.8 ± 0.1^{a}
	7	5.6 ± 0.6^{b}	$1.1 \pm 0.1^{a,b}$	0.6 ± 0.1^{a}	2.4 ± 0.2^{b}	3.7 ± 0.2^{a}
	10	$4.5 \pm 0.6^{a,b}$	1.6 ± 0.4^{b}	0.8 ± 0.1^{a}	1.6 ± 0.4^{a}	3.8 ± 0.6^{a}
	30	$4.5 \pm 0.6^{a,b}$	1.6 ± 0.4^{b}	0.7 ± 0.2^{a}	1.6 ± 0.4^{a}	3.8 ± 1.1^{a}
	60	3.8 ± 0.8^{a}	1.6 ± 0.4^{b}	0.7 ± 0.3^{a}	$1.7 \pm 0.6^{a,b}$	3.6 ± 1.2^{a}
Centrifugal mill	1	4.2 ± 0.9^{a}	0.9 ± 0.2^{a}	0.4 ± 0.1^{a}	1.9 ± 0.2^{a}	2.9 ± 0.3^{a}
	2	3.6 ± 0.3^{a}	0.9 ± 0.3^{a}	0.4 ± 0.1^{a}	2.1 ± 0.2^{a}	$3.1 \pm 0.1^{a,b}$
	5	4.2 ± 0.4^{a}	1.1 ± 0.2^{a}	0.5 ± 0.1^{a}	2.3 ± 0.2^{a}	3.8 ± 0.5^{b}
	7	3.6 ± 0.4^{a}	0.9 ± 0.2^{a}	0.4 ± 0.1^{a}	2.1 ± 0.2^{a}	$3.6 \pm 0.6^{a,b}$
Mincer	1	4.0 ± 0.8^{a}	1.4 ± 0.2^{a}	0.8 ± 0.2^{a}	1.5 ± 0.3^{a}	1.9 ± 0.7^{a}
	2	$4.9 \pm 0.9^{a,b}$	1.5 ± 0.2^{a}	$0.9 \pm 0.1^{a,b}$	1.8 ± 0.1^{a}	2.5 ± 0.6^{a}
	5	6.5 ± 0.9^{b}	1.5 ± 0.1^{a}	1.1 ± 0.1^{b}	1.7 ± 0.2^{a}	2.9 ± 0.7^{a}
	7	6.5 ± 0.8^{b}	1.6 ± 0.1^{a}	1.1 ± 0.1^{b}	1.7 ± 0.2^{a}	2.9 ± 0.7^{a}

Different letters (a and b) mean that there is a statistically significant difference ($p \leq 0.05$) with Duncan's multiple range test.

2021a; López et al., 2018). On the other hand, in the case of the mortar grinder, longer times were studied as this type of grinder allowed it, but no statistically significant differences were observed with the control (time 0), as shown in Fig. 2, neither between the other times as shown in Table 2.

3.1.2. *Effect of consecutive grindings*

The effect of grinding was evaluated with crank mills where the grinding time could not be controlled. However, consecutive grinds of 10 g of sample (maintaining the same portion of sample in each consecutive grind) were studied. These experiments (numbered 4 and 5) involved one specifically for poppy seeds and another designed for regular coffee grinding purposes. In the case of the poppy seed mill, it was possible to do 3 consecutive grindings, but in the case of the coffee mill, only 2 were possible. As shown in Fig. 3, no significant differences were shown between the consecutive grindings with the control (time 0), so no degradation trend was shown in any of the analytes.

However, if successive grindings are compared as shown in Table 3, statistically significant effects for morphine can be seen between the first and second grindings with both the poppy seed mill and the coffee grinder, with approximately 20% degradation of morphine. However, this effect was not observed for any of the other OAs. This could be because the oxidation of OAs primarily involves the formation of N-oxides, where oxidation occurs through the amino groups of these compounds, resulting a bond formation between the nitrogen (N) and oxygen (O) atoms. Nitrogen possesses unshared pairs of electrons that it can offer to oxygen. The accessibility of these electron pair on the nitrogen influences the ease of their donation, thereby determining the compound's susceptibility to oxidation. As shown in Figure S1, the pair of electrons within the N atom of codeine and thebaine exhibits increased delocalization, primarily attributed to the aromatic rings' double bonds in these compounds and their polar ether groups. While these groups are comparatively less polar than those of morphine, the structure of thebaine contains a higher number of conjugated double bonds, making it more difficult to oxidise. Conversely, papaverine and noscapine are more resistant to oxidation due to the nitrogen electron pairs are more delocalized. Papaverine, with its aromatic ring, is more difficult to oxidize than noscapine. Noscapine shares similarities with codeine and thebaine but poses greater difficulty in oxidation due to the presence of multiple polar groups such as ether, carbonyl, and aromatic rings, which collectively reduce the availability of the nitrogen electron pairs and thus increase resistance to oxidation (Figure S1).

The fact that some degradation is observed with this type of mill and not with the others could be due to the protection effect of the oil content of the seeds. In the first 3 experiments, the mechanical cracking releases some of the seed oil, producing a pasty product (as shown in Fig. 1). This oil is oxidised by oxygen, having a protective effect on the morphine, and preventing it from oxidising. But in experiments 4 and 5 this effect does not occur; a finely divided flour product is obtained because the oil is not extracted from the seeds and thus oxygen interacts with the morphine and oxidises it.

Besides, as in the previous experiments, possible differences were observed between the different studies (experiments 4 and 5). But this could be due to the high dispersion of OAs in the same bag (Carlin et al., 2020; Casado-Hidalgo et al., 2021a; López et al., 2018).

3.2. *Comparison of the results obtained in the present work with those published previously*

The results obtained from the various ground seeds showed no significant differences compared to the control (time 0). This suggests that there is no degradation effect after grinding. However, it is important to note that the dispersion in OAs concentration in poppy seeds can be quite high, even within the same bag, as demonstrated in previous studies (Carlin et al., 2020; Casado-Hidalgo et al., 2021a; López et al., 2018). This fact may lead to an absence of a significant degradation effect on the OAs concentration after grinding.

On the other hand, the results from each grinding condition were compared to observe potential degradation trends by subjecting the same sample portion to extended grinding times or consecutive grindings. Consequently, it was observed that mills like the poppy seed (experiment 4) or coffee grinder (experiment 5) can exhibit a statistically significant degradation effect reducing morphine levels by approximately 20% between the first and second consecutive grindings. However, no degradation trend was observed for the rest of the OAs. The influence of the milling type appears to be associated with oil extraction from the seeds. In milling methods where a larger portion of the oil was extracted, resulting in a paste, no degradation effect was observed. This lack of degradation can be attributed to the oil oxidation, preventing morphine oxidation when exposed to oxygen. This aligns with the rationale provided by EFSA in 2014 in their good practice guidance for reducing morphine concentration. According to EFSA, an increased presence of oxygen during seed grinding could lead to a 25–34% oxidation of morphine, forming degradation compounds such as pseudomorphine (European Commission, 2014).

Furthermore, a study conducted by Sproll in 2007, utilizing a poppy seed mill like the one used in this current work (experiment 4), discovered that morphine degradation could reach up to 34 ± 5% (Sproll et al., 2007). Likewise, Avula et al. 2023 similarly determined a potential 10% decrease in certain OAs like morphine, codeine, and thebaine. However, they did not specify the type of grinding method or its conditions (Avula et al., 2023).

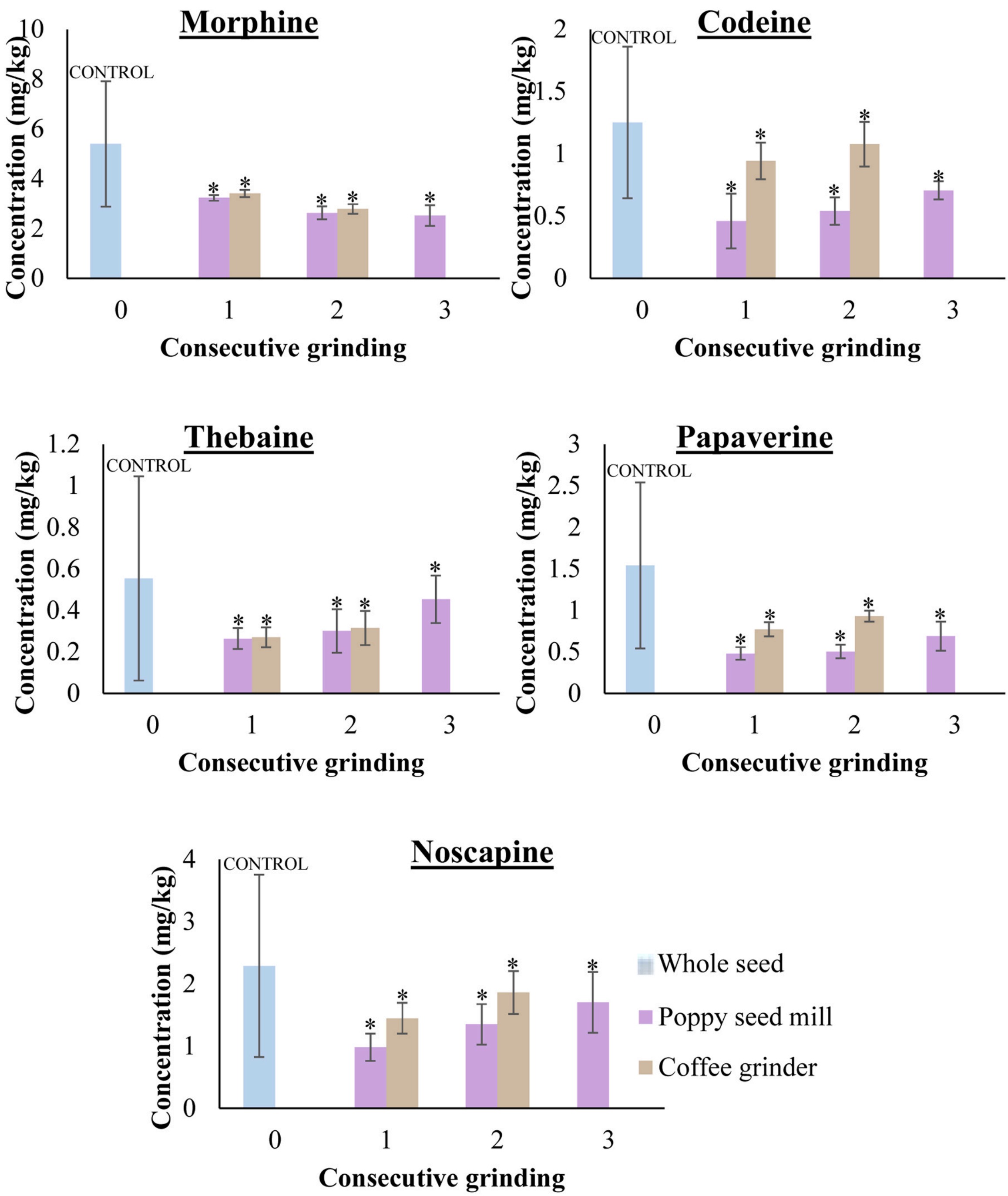

Fig. 3. Concentrations of each of the OAs determined in poppy seeds ground with different types of grinding (poppy seed mill and coffee grinder) with consecutive grindings (1, 2 and 3 times). * Mean that there are no statistically significant differences and ** there are statistically significant differences ($p \leq 0.05$) between the different times with control.

Table 3
Compilation of all the results obtained in each of the grinding studies which is possible to evaluate the consecutive grindings.

Grinding type	Consecutive grinding number	Morphine	Codeine	Thebaine	Papaverine	Noscapine
Poppy seed mill	1	3.2 ± 0.1^a	0.5 ± 0.2^a	0.3 ± 0.1^a	0.5 ± 0.1^a	0.9 ± 0.2^a
	2	2.6 ± 0.3^b	0.5 ± 0.1^a	0.3 ± 0.1^a	0.5 ± 0.1^a	1.4 ± 0.3^a
	3	2.5 ± 0.4^b	0.7 ± 0.1^a	0.5 ± 0.1^a	0.7 ± 0.2^a	1.7 ± 0.5^a
Coffee grinder	1	3.4 ± 0.1^a	0.9 ± 0.1^a	0.3 ± 0.1^a	0.8 ± 0.1^a	1.4 ± 0.3^a
	2	2.8 ± 0.2^b	1.1 ± 0.2^a	0.3 ± 0.1^a	0.9 ± 0.1^a	1.9 ± 0.3^a

Different letters (a and b) mean that there is a statistically significant difference ($p \leq 0.05$) in the case of poppy seed mill with Duncan test and in coffee grinder with Student t due to two levels.

3.3. Grinding type and conditions recommended according to results

According with the results obtained, the type of grinding employed can indeed impact the degradation of morphine, manifesting varying effects among them. Nevertheless, these degradation effects tend to exhibit a notably low percentage and do not seem to affect other OAs, which might exist in considerably high concentrations and possess potentially higher toxicity. It is imperative to recognize that grinding, as a culinary processing method, does not significantly induce OAs degradation. As such, relying solely on grinding to prevent or eliminate OAs in poppy seeds may not be effective. This process proves challenging to evaluate due to substantial variability in seed proportions within the same bag.

Based on the results obtained in the present study and those obtained previously by other authors, the type of grinding that should be performed on poppy seeds to minimize the content of OAs and thus ensure food safety is with crank mills, whether specific for poppy seeds or for coffee. The key to grinding to degrade certain morphine content is that there is no extraction of the oil that would protect the alkaloids from oxidation. In addition, the more consecutive grinds that are performed, the greater the degradation, so it should be recommended to perform at least two grinds to see this effect. However, it should be noted that the effect on the rest of the OAs is negligible and on morphine is minimal, so the ideal would be to combine this type of processing with others that have shown a significant degradation of the OAs, such as heat treatment (Vera-Baquero et al., 2022) or lactic fermentation (Casado-Hidalgo et al., 2023b), as demonstrated in prior studies. Additionally, alternate methods like washing, as mentioned by EFSA (European Commission, 2014; Sproll et al., 2007), can complement these approaches.

4. Conclusions

In the present work, the influence of grinding on OAs concentrations in poppy seeds was evaluated, since it is one of the most used culinary processes for poppy seeds. For this purpose, five types of grinders were evaluated for the first time, some with potential industrial use and others more commonly used in the household. All methods were evaluated for any significant degradation trends concerning increased grinding time or successive grinding, depending on the grinding type. Significantly, degradation effects on morphine were only observed with grinding methods that produced a finely divided flour product, without loss of oil from the seeds. This phenomenon might be attributed to the protective role of oil against oxygen-induced oxidation. Nonetheless, the results indicate that this processing approach should not be considered an effective practice for OAs reduction in seeds as the observed degradation effect is limited and only shown in morphine. Therefore, other types of culinary processing should be used to reduce the OAs content of seeds or research on combining grinding with other types should be carried out.

CRediT authorship contribution statement

Isabel Sierra: Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Damián Pérez-Quintanilla:** Writing – review & editing, Supervision, Investigation. **Sonia Morante-Zarcero:** Writing – review & editing, Supervision, Investigation. **Gema Casado-Hidalgo:** Writing – original draft, Validation, Investigation, Formal analysis.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jfca.2024.106098.

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