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Experimental determination of pesticide processing factor during extraction of maize germ oil

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Abstract – As there is a lack of regulations on pesticide levels in crude oils, this study aimed to evaluate transfer factors for extrapolating concentration limits established for maize kernels to the crude oils extracted from their germs. Maize kernels were spiked with 4 organophosphates insecticides (chlorpyriphos, chlorpyriphos-methyl, fenitrothion, pirimiphos-methyl), 2 pyrethroids (cypermethrin and deltamethrin) and a pyrethroid synergist, piperonyl butoxide (PB) targeting a dose of 1 mg/kg grain. The kernels were transformed in a pilot starch-mill to separate the germs (wet-milling) then these germs underwent an oil extraction process in a mini-pilot comprising a thermal conditioning stage, a mechanical extraction followed by a solvent extraction and a desolventization-toasting stage for the meal. Analyses were carried out by gas chromatography coupled to MS/MS detector. The pesticides content was measured on kernels, spiked kernels, germ, crude oil and meal. Surprisingly, a significant difference in behavior was observed between organophosphates and pyrethroids in the transition from grain to germ. For the former, the applied pesticides were recovered at levels between 48% and 81% in the germ, compared to only 9–11% for the latter and 31% for PB. It has been shown by means of acetonitrile washing on spiked seeds that the pyrethroids remained bound to the hydrophobic cuticle of the grains. On the other hand, from the germ to the oil, the initial hypothesis of an almost total transfer of the pesticides in the fat fraction of the material was confirmed. The germ to meal concentration ratios were on average 0.019, 0.065 and 0.109 for organophosphates, PB and pyrethroids respectively. In the same order, the concentration ratios between germ and crude oil were 1.87, 1.98 and 2.17. Grain to final oil transfer factors ranged from 7.4 to 12.7 for organophosphates, 4.8 for PB and 1.4 and 1.7 for cypermethrin and deltamethrin respectively.

Keywords: Organophosphorus / pyrethroids / transfer / spiking / GC–MS/MS

Résumé – Comme il n'existe pas de réglementation sur les teneurs en pesticides dans les huiles brutes, cette étude visait à évaluer les facteurs de transfert permettant d'extrapoler les limites de concentrations établies pour les graines aux huiles brutes de maïs. Des grains de maïs ont été dopés avec 4 insecticides organophosphorés (chlorpyriphos, chlorpyriphos-méthyl, fénitrothion, pyrimiphos-méthyl), 2 pyréthroïdes (cyperméthrine et deltaméthrine) et un synergiste des pyréthroïdes, le piperonyl butoxyde (PB) en visant une dose de 1 mg/kg de grain. Les grains ont été transformé dans un atelier pilote d'amidonnerie pour en séparer les germes (procédé voie humide) puis ces germes ont subi un procédé d'extraction de l'huile en mini-pilote comprenant une étape de conditionnement thermique, une extraction mécanique suivie d'une extraction par solvant et une étape de désolvantation-toastage pour le tourteau. Les analyses ont été effectuées par chromatographie en phase gazeuse couplée à détecteur MS/MS. Les teneurs en pesticides ont été mesurées sur grain, grain dopé, germes, huile brute et tourteaux. De manière surprenante, il a été observé une différence importante de comportement entre organophosphorés et pyréthroïdes pour ce qui est du passage du grain au germe. Pour les premiers, les pesticides appliqués se retrouvent de 48 à 81 % dans les germes contre seulement 9–11% pour les seconds et 31% pour le PB. Il a pu être montré au moyen de lavage à l'acétonitrile sur graines dopées que les pyréthroïdes restaient fixés sur la cuticule hydrophobe des grains. En revanche, du germe à l'huile, on confirme l'hypothèse initiale d'un transfert quasi-total des pesticides dans la fraction grasse de la matière. Les rapports de concentration germe *versus* tourteau étaient en moyenne de

0.019, 0.065 et 0.109 pour organophosphorés, PB et pyréthroïdes respectivement. Dans le même ordre, les rapports de concentration entre germes et huile brute étaient de 1.87, 1.98 et 2.17. Du grain à l'huile finale des facteurs de transfert étaient compris entre 7.4 et 12.7 pour les organophosphorés, 4.8 pour le PB et 1.4 et 1.7 pour cyperméthrine et deltaméthrine respectivement.

Mots clés : organophosphorés / pyréthrinoïdes / transfert / dopage / GC–MS/MS

1 Introduction

The production of corn oil is generally derived from the wet milling industry. The dry process is mainly dedicated to ethanol production where the germs are not recovered and end up in the co-product called "dried distillers' grain with solubles" or DDGS (Shad, 2021). Maize processing by wet milling involves 7.5 Mt of grain per year in the European Union (Starch Europe, 2023). It is responsible for marketing 264 kt/year of maize germ oil for Europe-28 and 378 kt for the entire continent, and about 3Mt worldwide, including 1.74 Mt for the Americas (FAOSTAT, average 2016–2020). Within the 28-member Europe, corn oil consumption accounts for only about 1% of all vegetable oils (~27Mt, including rapeseed 34%, palm 25%, sunflower 18%, soybean 9% and olive 7%, sources: FEDIOL, 2023; International Olive Council, 2023).

This work is the continuation of a previous study made by ITERG and OLEAD to experimentally determine the processing factors of post-harvest insecticides during the processing of rapeseed (Lacoste *et al.*, 2020). The same methods were used for the extraction of maize germs, starting from corn kernels spiked with a selection of 7 molecules which were of major concern regarding the contamination of maize germs oil (MGO). Unlike oilseeds, maize and cereals are subject to the possibility to receive post-harvest insecticides. At the time the study was designed, maximum residue levels (MRL) for Chlorpyriphos, Chlorpyriphos-methyl, Cypermethrin, Deltamethrin, Fenitrothion, Pirimiphos-methyl were respectively 0.05, 0.05, 0.3, 2, 0.05, 0.5 mg/kg. Since 2020, the regulation has moved toward more stricter thresholds for chlorpyriphos and chlorpyriphos-methyl, (0.01 mg/kg) (source UE database on pesticides, 2023). Piperonyl-butoxide which is a synergistic agent that reinforce the effects of pyrethroids without direct toxicity has no MLR but being a more stable molecule than pyrethroids, it is considered as a marker of possible previous insecticide treatment.

On pesticide residues, FAO and WHO collaborate in the Joint Meeting on Pesticides Residues (JMPR), which issues recommendations on residues in raw materials. These recommendations are based on known transfer factors. The latter frequently come from studies carried out by phytosanitary companies which focus mainly on residues after field applications and are based on low doses limiting the reliability of the results.

Table 1 summarizes the available data on transfer factors for wet maize processing found in the monographs gathered by these organizations. Of our 7 molecules, only 3 have relatively complete data and for two of these molecules, these results come from a single study about the combination deltamethrin + piperonyl butoxide. The results obtained for PF are not in line with expectations since the concentration levels observed are far from following the concentration effect in the lipid phase that applies to oilseeds. It is particularly surprising not to see a concentration of pesticides in the germ fraction for the study on deltamethrin +piperonyl butoxide while the concentration in the oil fraction increases. This can be explained by several factors such as the low level of initial concentrations, which can lead to analytical difficulties or methodological problems. Cho *et al.* (2021) in a literature review also cited a similar result for another organophosphate: dichlorvos with a PF of 1.2 from grains to crude oil.

In the state of knowledge, we know that pesticides applied in the field are found only at low concentrations in grains. Thus, in the JMPR monograph on chlorpyrifos, residues below LOQ (0.01 mg/kg) are mentioned for field applications at doses up to 2.2 kg of active ingredient per hectare. According to Holland *et al.* (1994), hydrophobic insecticides applied to oilseeds crops at field level are found at very low concentrations in seeds. The only pesticides of real concern are those applied for seed preservation in storage. According to these authors, insecticides of this class are often attached to the seed husks and migrate only partially to the lipid reserves of the germ. This is the case for cotton seeds protected by linters whose transfer factor from seed to oil is of the order of 0.8 for cypermethrin. Mahugija *et al.* (2017) examined concentrations found in maize seeds and flours found in markets or mills prior to polishing in Tanzania. It appears from this work that the processing of flours, which eliminates a large part of the bran, tended to significantly reduce insecticides concentrations. Thus, methyl-pirimiphos which is the most frequently encountered organophosphorus is found in 55% of seed samples at an average concentration of 1.82 mg/kg (seeds with residues), 87% of flour samples before polishing with an average concentration of 0.73 mg/kg and 41% of flour samples at retail level with 0.08 mg/kg commercial flour with residues. The increase in frequency and decrease in the concentration of flour before polishing can be explained by the dilution of batches with residues in the mass of non-treated material, the decrease in the concentration in flour after polishing by the higher concentration of organophosphates in the bran.

In another area, olive oil, a 2018 study (López-Blanco *et al.*) investigated processing factors relating to 108 pesticide molecules with different levels of affinity for lipids. Their objective was to verify the correctness of an assessment of MLRs in oil as a function of MLRs in olives, log $P_{o/w}$ values, and extraction yields. Overall, they observed a fairly low R^2 (0.59) between the predicted values and the PFs actually measured. They were thus able to establish that the slopes followed by the clouds of dots depended on the families of molecules. For the organophosphate family, they obtained an R^2 of 0.8 for log $P_{o/w}$ values ranging from −0.8 to +5. The regressions obtained improve the quality of the prediction based on octanol/water partitions. Zincke *et al.* (2022) of the BfR (German Federal Institute for Risk Assessment) were mandated by EFSA to update the database on Processing Factors. This database, which was commissioned in 2016,

Table 1. Available data about the evolution of pesticides residues during the production of corn/maize oil.

Molecule	Kernel (mg/kg)	Germ (mg/kg)	Crude oil (mg/kg)	Processing factor	JMPR reference
Chlorpyriphos Dry milling (Illinois) (Michigan) Wet milling (Illinois) (Michigan)	 0.01 0.04 0.01 0.04		 0.01 0.06 0.01 0.12	 1 1.5 1 3.0	2009
Chlorpyriphos-methyl (France) (Wheat, Australia)	<LOQ 5.7	 23.8	<LOQ	 4.2	1993 2009
Cypermethrin (no data for corn/maize) (Wheat – grain à bran) (Wheat – grain à germ)				 2.4 0.56	1990
Deltamethrin Wet milling 42 days if storage Wet milling 182 days storage	 0.263 0.583 0.111 0.362	 0.005 0.022 0.000 0.000	 0.010 0.599 0.143 0.455	 0.019/0.038 0.04/1.03 0.00/1.3 0.00/1.3	2002
Fenitrothion (no data for corn/maize) (Wheat grain à bran after 1 month storage) (Wheat grain à germs 1 month storage)	 7.0	 (bran) 28.0 26.0		 4.0 3.7	2003
Piperonyl butoxide (Italy) Wet milling 42 days if storage Wet milling 182 days storage	 1.05 3.7 0.8 2.2	 <0.1 <0.1 0.13 0.71	 <0.1 <0.1 <0.1 <0.1		2001
Pirimiphos-methyl (no relevant data)					2003

required an update. 1301 process studies were added, more than doubling its size. An important addition concerned palm and palm kernel oils. A recent consultation of this database (EFSA, 2023) shows that it contains 5725 data among which there is only one reference crossing one of the 7 substances studied in this study and maize. It refers to a study that failed to establish PF for this occurrence (EFSA, 2012).

The EU vegetable oil and protein meal industry association (FEDIOL) commissioned this study to supplement the available data with the case of MGO which differs from common oilseeds by the low concentration of oil in the maize kernels. FEDIOL proposes a method to assess the MRLs in crude vegetable oils and fats to fill a gap in the European regulation which specifies limit values in seeds but not in crude oils. This method considers that highly hydrophobic contaminant, molecules with log P_{ow} >3, are totally migrating in the oil and therefore estimates MRL with a factor related to the oil content of the seeds. For example, rapeseed with 42−45% of oil, it is safe to use a 2.5 processing factor to predict the concentration of a contaminant in crude oil starting from the concentration in the seed. Thanks to this criterion, it becomes possible to know if the molecule concentration in crude oil is above or below MRL. This approach is proposed to overcome the lack of regulation concerning crude oils contaminants levels, especially to regulate trading between crushers and refiners by giving them rules for the determination of acceptable levels of residues.

The previous study was made with spiked seeds which were processed at the scale of 10 kg by a sequence of unit operations reproducing industrial conditions in terms of temperatures, moisture, duration and extraction methods (mechanical extraction followed by hexane extraction). Briefly, seeds were sprayed with a mixture of 4 insecticides and 1 fungicide to obtain pesticides concentrations in seeds from 0.98 to 1.39 mg/kg. The seeds were stored for 2 weeks before processing. After flaking, the seed were cooked to reach a final temperature of 95°C in 40 minutes. The cooked material was then pressed to give press oil and press cake. The press cake was then extracted with technical hexane and the solvent was removed from the resulting marc and miscella to give oil which was mixed with the press oil and meal which was toasted for 80 min in order to reach 105°C while sparging direct steam. The pesticides residues were retrieved almost exclusively in the oil fraction for the 4 insecticides

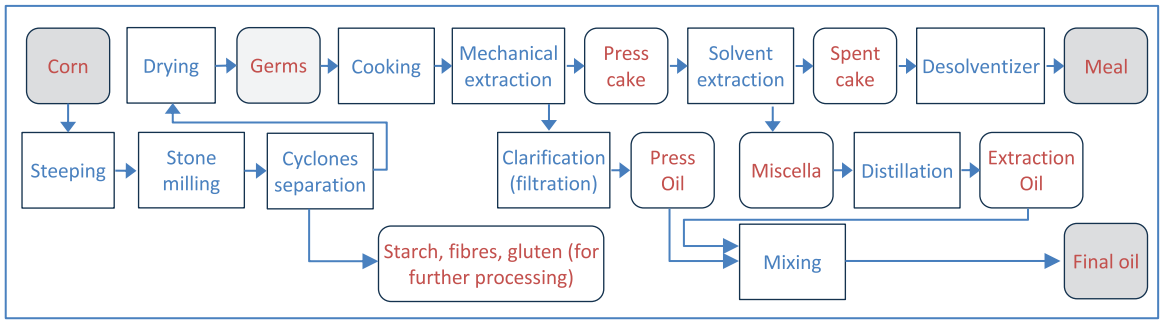

Figure 1. Flowchart representing the processing steps reproduced in this study.

Table 2. List of the pesticides used for the spiking including batch ID and expiration dates.

Pesticides	CAS	Supplier	LOT	Purity	Expiration
Chlorpyriphos	2921-88-2	HPC	790083	99.3%	01/07/2024
Chlorpyriphos-methyl	55598-13-0	HPC	787202	99.6%	01/02/2025
Cypermethrin	52315-07-8	HPC	789356	99.7%	01/03/2024
Deltamethrin	52918-63-5	HPC	780147	99.7%	01/06/2022
Fenitrothion	122-14-5	HPC	777942	94.8%	01/11/2021
Piperonyl butoxide	51-03-6	HPC	781797	94.4%	01/08/2023
Pirimiphos-methyl	29232-93-7	HPC	782161	98.5%	01/12/2022

characterized by log $P_{ow} > 3$ while the fungicide with a log P_{ow} of 1.71 was retrieved a little more in the meal. The results were a net confirmation of the FEDIOL proposal to consider that hydrophobic molecules are migrating in the oily compartment of the seeds and that good approximation of their concentration in the crude oil can be extrapolated from the oil content of the seeds.

MGO processing as mentioned earlier differs from other seeds oils by further processing steps required to extract the germs from the kernels. After steeping, the maize is dislocated in disk mills to fractionate the components. The slurry resulting from this operation is separated in hydrocyclones where the germs which with 40−50% of oil have a lower density and can be recovered in the overflow. After further steps of purification in hydrocyclones, the germs are dried to be processed in the same way than common oilseeds (Fig. 1). Considering that these processing steps are involving prolongated contact with water and that germs recovery could be only partial, processing factors were likely to be different from other oilseeds. On the other hand, if the processing factor in the case of MGO could be proved consistent, that would enhance the robustness of the method and increase the confidence of stakeholders in its assessments.

2 Materials and methods

2.1 Corn grains spiking

In principle, the method of doping a grain sample with pesticides by spraying is not representative of what happens in reality for vegetation-applied pesticides. However, in the case of this study, since it concerns post-harvest insecticides, the objection can be overcome, and the method was considered acceptable. 500 kg of commercial corn was supplied by the Cargill company. The kernels origin was non-specified, the harvest year was 2018 and the processing occurred in the August 2019 for the grain spiking, November 2019 for the germs extraction and December 2019 for the crude oil extraction.

The list including 7 molecules is presented in Table 2. These molecules are used to manage pest development in harvested seeds. Among these molecules, four (chlorpyriphos, chlorpyriphos-methyl, fenitrothion and pirimiphos-methyl) were organophosphorus, two (cypermethrin and deltamethrin) were pyrethroids, and one (piperonyl butoxide) was a synergist of pesticide with no direct pesticide properties in itself but used to enhance the efficiency of certain insecticides by inhibition of enzymes involved in the degradation of active molecules (Moores *et al.*, 2009). Testing the pyrethroids together with piperonyl butoxide was never tried but it was assumed that it has no effect regarding processing factors. A primary solution was prepared with the 7 molecules to be mixed with the maize grains with a spiking target of 3 mg/kg.

The solution was prepared by weighting 1.9 g of each pesticide exactly into a 50 mL flask and then making up to the mark with acetone. Each flask was homogenized for at least 15 seconds, 1 mL of each stock solution was transferred into a vial and the remaining 49 mL of each stock solution was poured to the same 500 mL flask (Diluted Standard Solution, DDS). Stock solution flasks were rinsed with acetone to recover all remaining pesticides and finally, the volume of the 500 mL DDS flask was adjusted with acetone and then manually homogenized for 30 seconds. Ten 200 mL vials were prepared with 40 mL volumes of DDS (Table 3) and 110 mL of acetone. The method differs from practical application of pesticides where the commercial product contains formulating agents like surfactants helping the active molecules to cross the grain

Table 3. Preparation of the diluted standard solutions (DSS).

Molecule	Volumes DDS	Mass in each batch (mg)
Chlorpyriphos	40 mL	147.96
Chlorpyriphos-methyl		148.36
Cypermethrin		148.04
Deltamethrin		149.01
Fenitrothion		142.42
Piperonyl butoxide		140.86
Pirimiphos-methyl		146.96

coats and improve adhesion on the surface. Nevertheless, using acetone as solvent seemed a good alternative to facilitate the dispersion of the pesticides and a regular spreading on the grain surface. The vials were homogenized by manual agitation and stored at −20°C.

The official spiking protocol for grains, *i.e.*, CEB 106 (Commission des Essais Biologiques) was undertaken from 10 to 13 September 2019 by the laboratory SITONA AgroExpert. Fifty kilograms of maize were placed into a rotating device and air-sprayed with 150 mL of the prepared solution. The operation was repeated 10 times to obtain a batch of 500 kg of spiked kernels. Ambient temperature ranged between 12.8 and 18.5 °C. Spraying duration was comprised between 84 and 96 seconds. The corn kernels were packed in plastic bags of approximately 17 kg and the bags placed in 1 large carboard box for storage and shipping. The shipping toward Vilvoorde's Cargill facility was made in first days of October 2019. The germs were ready to be shipped back to Pessac (place of ITERG facilities) on mid-November 2019.

2.2 Germs extraction and purification

Figure 1 represents a general overview of the processing that was reproduced by this study. At the Cargill pilot plant, the kernels were processed according to the wet-milling method including stepping for 27 h in presence of sulphur dioxide to prevent biological evolution of the solution, the kernels were milled using a stone mill with two passes in order to separate the germs from the rest of the material and the germs were recovered by manual separation on using a meshed tool and washed aiming at maximizing the purity of the germs fraction. The final step was a drying at 70−72°C for 12−15 h in a laboratory oven on perforated plate, the objective being a water content below 5%.

2.3 Germs and oils processing

The germs were delivered in three batches of about 4 kg. The processing was carried out separately for each batch.

A thermal conditioning was carried out in a bench-cooker composed by a stainless-steel vertical cylinder (diameter 220 mm), heated from the bottom by a 1000 W resistor and stirred by a blade scrapping the heating surface and a vertical spiral tape. The heating kinetic was adjusted to reach 100−105 °C in approximately 15 minutes and then, the temperature was maintained for 30 minutes. This kinetic was controlled by adjusting the tension at resistor terminals. Due to the size of the bench cooker, the operation was made in two time for each batch of germs. At the end of the treatment, the germs were taken out of the cooker and spread in a thin layer for rapid cooling.

The germs were mechanically extracted using a Komet CA59G screw press fitted with a 6 mm nozzle at 20 revolution per minute. Due to the low moisture content of the germs (2.27, 0.87 and 0.89% for respectively batches 1 to 3), a moistening step was carried out before extraction to get water contents of approximately 5 % before mechanical extraction. The press oil was filtered using a paper filter on a vacuum funnel (Buchner). Press cake was hexane extracted in a 6 L percolation column (diameter 135 mm, in-house fabrication) by 4 washes, each using 5 L of solvent. The extraction column has a removable perforated bottom and a double jacket where hot water is circulated (55°C). The solvent is circulated by a membrane pump through a heat exchanger and can be returned to the extraction column for the desired duration. For each washing, the solvent was preheated at 50°C. Each extraction step lasted 10 minutes. At the end of the extraction, a measure of percolation speed was performed by measuring the time required to get 2 L of miscella while the column was submerged by the miscella. This measure is required to check the quality of the extraction. At the end of extraction time, the marc was allowed to drain up to the moment were miscella stops dripping from the extractor, the miscella was then evacuated and a new volume of fresh solvent was introduced for the following wash. The final marc was discharged and spread on a tray placed under a laboratory fume hood to let the solvent evaporate.

The solvent was evaporated from the miscella in a rotary vacuum evaporator (Hei-VAP Advantage, Heidolph, Germany). The solvent extraction oil was mixed with the press oil for subsequent analysis.

The spent cake (meal) was subjected to a new passage in the bench cooker to reproduce the thermal treatment operated in the industrial desolventizers (105°C, direct steam, 80 min).

2.4 Grain washing

4 g of spiked kernels were mixed with 100 mL of solvent for 10 minutes at ambient temperature and agitated with an orbital device (600 pulses/min). After the wash, the solvent was poured out of the balloon and the kernels retained on a paper filter (glass fibre), 50 mL of solvent was used to rinse the kernels and the

Table 4. *Parameters for quantification of pesticide residues and internal standards by MS/MS.*

Pesticides	Molecular weight (g/mol)	Retention time (min)	Quantifier (Quantification ion) (*m/z*)			Qualifier 1 (Quantification ion) (*m/z*)			Qualifier 2 (Quantification ion) (*m/z*)			Qualifier 3 (Quantification ion) (*m/z*)			Qualifier 4 (Quantification ion) (*m/z*)		
HCH-alphaD6	296.9	16.42	223.8	→	186.9	186.9	→	149.9	225.9	→	188.9						
Chlorpyrifos-methyl	322.5	20.18	263.8	→	168.0	263.8	→	229.0	265.8	→	231.0						
Pirimiphos-methyl	305.3	21.37	265.0	→	220.0	265.0	→	93.0	250.0	→	220.0						
Fenitrothion	277.2	21.43	273.7	→	238.9	273.7	→	236.9	234.0	→	174.1	271.7	→	236.9			
Malathion D7	337.4	21.73	290.0	→	125.0	234.0	→	146.1	220.0	→	192.1						
Chlorpyrifos	350.6	22.03	109.0	→	79.0	125.1	→	79.0	277.0	→	109.0						
Piperonyl butoxide	338.4	29.71	235.1	→	165.1	237.1	→	165.1	235.1	→	199.1						
Cypermethrin	416.3	36.08	163.0	→	127.0	163.0	→	91.0	183.0	→	165.1	183.0	→	153.1			
Deltamethrin	505.2	38.68-39.06	252.9	→	93.0	208.2	→	181.1	181.2	→	127.1	250.7	→	172.0	181.2	→	152.1

residual solvent was evaporated overnight at room temperature, the kernels being allowed to rest in fume cupboard.

2.5 Statistical analysis

Mean comparisons using *t* test were done using the function "T.TEST" of Excel software, using unilateral comparison and unequal variances.

2.6 Analysis

Pesticides residues analysis: The pesticide analysis was made in 3 steps: 1) pesticides extraction with acetonitrile; 2) purification by QuEChERS method (adsorbent); 3) concentration under nitrogen and injection in GC−MS/MS. Briefly, a 1 g sample portion, together with added internal standard solution, are extracted twice with 6 mL acetone under automated vortex stirring for 5 minutes. After centrifugation, the supernatants are pooled, and, after 10 seconds vortex stirring, 8 mL are transferred into a QuEChERS tube (dSPE 15 mL for AOAC 2007.01–1200 mg $MgSO_4$, 400 mg PSA, 400 mg C18 (Restek Ref 26221 or equivalent). After 5 min stirring under vortex agitation and centrifugation, 4.5 mL of the purified extract is evaporated under Nitrogen flux at about 35°C. Finally, the resulting concentrated 200 µL extract is analyzed (Table 4).

Quantification was carried out by external calibration (8 pesticides range points) and using deuterated internal standards (Table 5). To ensure good repeatability and reproducibility of the results, a solution of "protectants" was added to the calibration points and to the samples to minimize the effects of the chromatographic system. It consisted of 500 mg 3-nonanone, 500 mg Trans 3 Hexan-1-ol, 100 mg cholesterol, 100 mg alpha-tocopherol, 500 mg hexanoic acid, 100 mg oleic acid and 500 mg glyceryl trioleate in a 10 mL graduated flask, filled with Toluene. 20 µL of the obtained solution is added into samples and calibration standard solutions.

2.6.1 Quantification

Selection of internal standards: The method here described is an extrapolation of an internal (ITERG) existing method under COFRAC accreditation that allows for the determination of more than 80 different pesticide molecules. Each quantified pesticide molecule is associated with one of three internal isotopic standards (alpha-HCH D6, malathion D7 et DDT D8). The association was optimized to minimize the total number of isotopic standards needed to quantify all the pesticide molecules, thereby warrantying appropriate recovery yields (70−120%) and repeatability/reproducibility (Cochran and Grubs test).

2.6.2 Other analyses

- Oil content for oilseeds without hydrolysis: NF EN ISO 11085
- Oil content for grains/hydrolyzed material: NF EN ISO 659
- Oil content of press cake and meals: NF EN ISO 734

Table 5. Nature of the internal standard for each pesticide molecule.

Pesticide	Internal standard
Chlorpyrifos	Malathion D7
Chlorpyrifos-methyl	α-HCH D6
Cypermethrin	α-HCH D6
Deltamethrin	Malathion D7
Fenitrothion	Malathion D7
Piperonyl butoxide	Malathion D7
Pirimiphos-methyl	Malathion D7

3 Results and discussion

3.1 Germs processing

3.1.1 Wet milling and germs recovery

The processing of the germs produced 3 batches of germs of 4.79, 4.06 and 4.33 kg, *i.e.*, a global yield of 2.6% which is below industrial performance (6−7%, Fox *et al.*, 1992; Deepak *et al.*, 2022). This low yield is explained by the pilot conditions of the processing which was optimized to maximize the purity of the germs and therefore allowing losses during the purification steps.

The average oil content of corn germs given by the database Feedipedia (Heuzé *et al.*, 2015) is 48.3% (DM) *versus* 48.9% (DM) in this work confirming the high degree of purity obtained. The oil content of the kernels was 3.7% after HCl hydrolysis (NF EN ISO 659) or 3.1% without hydrolysis (NF EN ISO 11085). These values are rather low by comparison with regular maize grain harvested in France (4.2/3.5% with and without HCl hydrolysis; Feedbase). According to Rajendran *et al.* (2012), about 83% of the kernel oil is located in the germs.

3.1.2 Mechanical extraction and preliminary cooking

Cooking. Figure 2 shows the temperature records of the germs during the thermal treatment. During the last 30 minutes of the treatment, the temperatures were comprised between 100 and 104°C. That treatment reproduces the conditioning of the material prior to the mechanical extraction. It leads to important modification in the ultrastructure of the cells organelles, especially at the level of oil-bodies which membrane is destroyed, oleosin proteins coagulate and phospholipids are partially solubilized in the oil (Prior *et al.*, 1991; Ponne *et al.*, 1996). The treatment is likely to modify the repartition of the pesticides in the material because oil is freed from the oil-bodies, and this enhance the likelihood to solubilize fats soluble material.

Moisturizing. Mechanical extraction has been requiring a moistening of the germs after the cooking because the water content after the cooking step was too low for allowing functional mechanical expression of the oil. Water has a plasticizing effect in the cake, allowing it to agglomerate and improving its compressibility. On the other hand, too much plasticity reduces the press ability to generate pressure because plasticity enhances creep, facilitating the flow of the solid through the press orifice (Carré, 2022). For this moistening, the water content of the germ was determined using a scale fitted with an infrared desiccator to determine the quantity of water required to reach 6% in the germs prior to mechanical extraction. Water was sprayed on the germ with a hand sprayer while the germs were homogenized using a rotary device used for measuring pellet durability according to the standard ISO 17831-1.

Mechanical extraction.

Table 6 reports the results of the cooked germs expelling. Although the pressing conditions were all the same for each batch (rotating speed 30 rpm, orifice diameter 6 mm, no heating of the press head), the results were quite different for each batch both for oil yield and, in a lesser extent, to the temperature of the cake. Oil yield and residual oil in the cake are perfectly coherent ($O = 67.3–1.32\ Y$, $R^2 = 0.999$; O, oil residues of the press cake; *Y*, unfiltered oil yield). Unfortunately, the water content of the germs before expelling and of the cake were not measured leaving no mean to confirm the putative explanation of these differences by unequal moistening of the batches. This confirms the sensitivity of mechanical extraction to minor differences in material plasticity as discussed by Carré (2022).

The crude oils were filtered immediately after obtention on a paper filter resulting in separation of clarified oil and filtration residues. The proportion of residues were 9.4, 12.5 and 9.0% for respectively batches 1, 2 and 3. The oil content of the filtration cakes were 41.5, 40.6 and 44.9% for batches 1 to 3.

Solvent extractions

According to the measure of the oil residues in the meal (Table 7), the solvent extraction was resulting in satisfactory oil recovery. On the other hand, the examination of the mass balance is much less satisfactory because based on oil content of the press-cakes, the oil yields (oil recovered/potential mas of oil in the cake) were 83.2, 94.6 and 136.5 % for the batches 1 to 3. Losses (mass of cake − mass of oil − mass of meal) were respectively of 201 g, 118 g and 89 g for batches 1 to 3. For batch 1, it seems clear that some oil was lost, likely during the miscella evaporation. A problem related to foaming during the last steps of the solvent evaporation should have led oil loss which probably ended up in the recovered solvent used for the extraction of the third batch. This would explain the excess of oil recovered in this batch. The oil mass transfer from batch 1 to batch 3 is of about 60−90 g of oil.

Meal toasting. After extraction the spent cake soaked with solvent was spread under a laboratory fume for allowing the evaporation of solvent to mimic the thermal treatment of the desolventization, the cakes were toasted with the bench cooker. For this operation, all the dried spent cake was placed in the cooker. The cooking time was set at 60 minutes including a 40-minute steam injection. This injection was cut a few minutes before the end of cooking. This steam injection was reproducing the toasting conditions of the industrial desolventization.

Figure 3 shows the temperatures registered during the toasting starting at the time where the temperature reached 70°C.

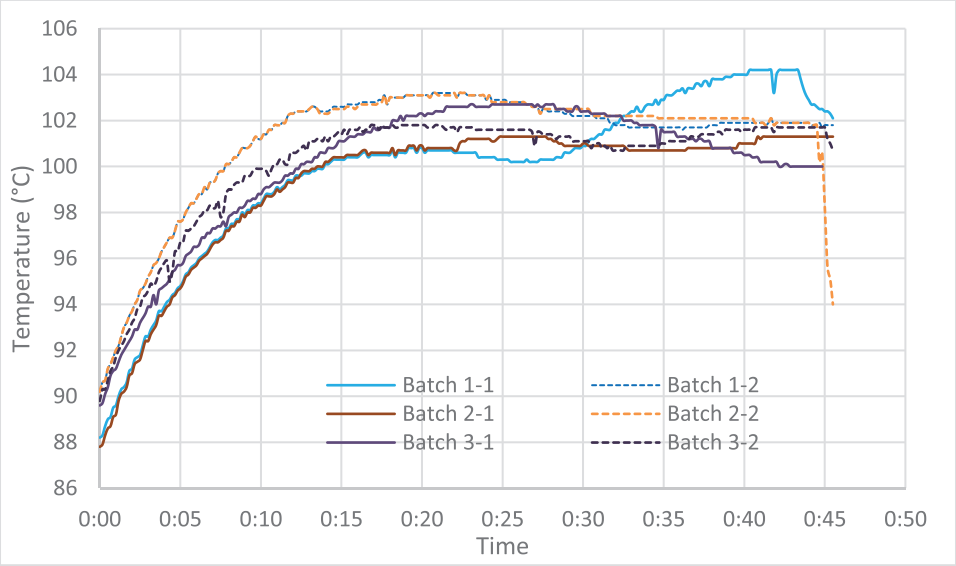

Figure 2. Temperature recording during heat treatment of the germs. Batches 1 to 3 were divided in 2 sub-batches because of the volume capacity of the bench cooker.

Table 6. Characteristics of the press behavior in germ processing.

Characteristics	Batch 1	Batch 2	Batch 3
Initial germ mass (g)	4348	3610	3830
Press cake mass (g)	2981	2568	2355
Unfiltered oil (g)	1309	992	1210
Flow rate (g/h)	3120	2810	2095
Unfiltered oil yield	31.4	27.8	38.0
Temperature of the cake (°C)	49.7	52.3	67.8
Temperature of the oil (°C)	37.3	37.0	50.7
Oil content of the press cake (%)	25.7	30.7	17.2

The duration for going from 70°C to 105°C between was comprised between 12 and 20 min. This temperature was maintained till the end of the treatment. Direct steam flow rate was 25 g/min.

3.2 Pesticides concentration

3.2.1 Pesticides dosage before and after spiking

A sample of each of the 10-maize kernel lots before and after spiking were analyzed (2 rep). Table 8 shows the results obtained before and after spiking.

The data preceded by ± sign represent the standard deviation of the concentration of the replicates except for the "Mean 10 samples" line where the standard deviations between batches were indicated. Variability between the batches is partly explained by the order of the treatments. Batches 1 to 6 and batches 7 to 10 were statistically different as it is shown in Fig. 4 for each molecule except for cypermethrin. The difference is probably explained by a change in the spraying which were made sequentially.

Knowing that spiking was made to get approximately 3 mg/kg of the pesticides at the level of the corn kernels, it is possible to estimate a losses rate after calculating the theoretical concentration from the doses that were used for nebulization. The theoretical dose is the initial mass of pesticide detected in the corn kernel before spiking to which is added the mass of pesticide from the spiking solution relative to the treated mass of kernels. Losses are calculated by dividing the difference between the theoretical and observed concentration by the theoretical concentration.

Losses are in the 57−68% range for organophosphate insecticides, 51−56% for pyrethroid and 24% for piperonyl butoxide. There is no clear relationship between the molar mass of the molecules and losses. Therefore, no satisfactory explanation can be given to these losses differences.

The kernels before spiking were already contaminated with three of the study molecules: piperonyl butoxide (1410 µg/kg), chlorpyrimiphos-methyl (261 µg/kg) and deltamethrin (140 µg/kg). A strong correlation can be observed between concentrations of piperonyl butoxide and deltamethrin concentrations before spiking (R^2=0.99). This correlation is not surprising since these molecules are formulated in the same commercial insecticide with a 10:1 ratio. The correlation between piperonyl butoxide and chlorpyrifos-methyl was weaker (R^2=0.85) but still

Table 7. Characteristics of the extraction by percolation of the press-cakes.

Characteristics	Batch 1	Batch 2	Batch 3
Mass of cake (g)	2775	2365	2155
Mass of oil (g)	594	687	506
Mass of meal (g)	1980	1560	1560
Oil content of the meal (%)	1.4	1.5	0.4
Oil yield (% oil/press cake)	20.6	28.2	24.6
Miscella concentration 1^{rst} wash (%)	18.2	17.6	17.8
Miscella concentration 2nd wash (%)	5.7	3.7	4.5
Miscella concentration 3rd wash (%)	1.8	0.6	0.5
Miscella concentration 4th wash (%)	0.1	0.1	0.1
Average extraction temperature (°C)	51.6	51.9	51.3

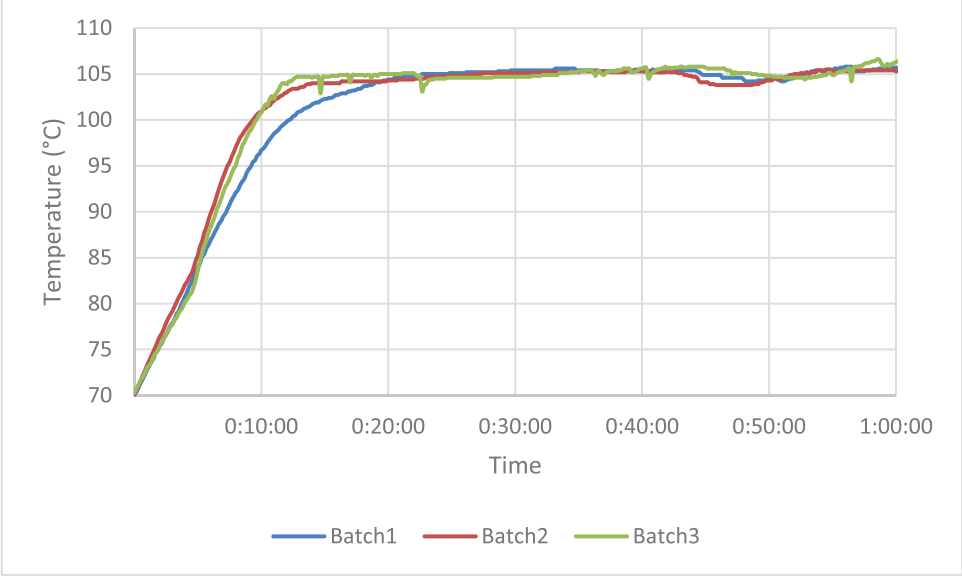

Figure 3. Temperature recorded during the toasting of the meals.

significant. It is therefore likely that the 500 kg maize kernel batch used in the study was containing an uneven part of kernels that received 2 applications of insecticides formulations.

3.2.2 Pesticides transfer from the kernels to the germs

Table 9 gives the pesticides concentrations in the kernels and the germs and the ratio between these concentrations (processing factor).

Measured concentrations in germs are below expectations in regard of the hypothesis where almost each pesticide with a log $P_{o/w}$ >3 goes in the oil fraction of the material. The log $P_{o/w}$ given in Table 10 are well above that threshold. Compared to literature data (Table 1) the processing factor for germs in this study are significantly higher, especially concerning deltamethrin where a value of 0.04 was found for kernels after 42 days of storage and 0.58 mg/kg of the molecule in the grain. Nevertheless, this study was inconsistent since the oil resulting from these germs was containing 0.60 mg/kg of deltamethrin. On the second hand, several studies related to dry milling exhibit significant concentration of the hydrophobic insecticides in bran and germs. That is the case for the samples of wheat mentioned in Table 1 for chlorpyriphos-methyl where a PF of 4.2 was found from grain to germs. With cypermethrin, the wheat brans were retaining more pesticides than the germs (PF 2.4 and 0.56 respectively). Fenitrothion also exhibited slightly higher retention of residues in bran than in wheat germs (PF of 4.0 and 3.7). Similar results were found by Mahugija *et al.* (2017) in corn and refined flour where elimination of the bran reduced the pesticides concentration.

In this work, it is possible to assess a theoretical mass balance of pesticides fate based on the concentration in the germs and a theoretical germ yield of 6.4%. Table 9 gives these masses by molecule for the kernels and for the germs. The recovery yields were relatively close to the expectations for the organophosphate family although fenitrothion with a lower log $P_{o/w}$ value was less transferred to the oil rich germs. It is not the case for piperonyl butoxide with a 31% yield and even worse for the pyrethroids with recovery yields of about 10%.

Table 8. *Concentration of each molecule in µg/kg measured before and after spiking and losses assessment.*

	Chlorpyriphos-methyl		Fenitrothion		Pirimiphos-methyl		Chlorpyriphos		Piperonyl butoxide		Cypermethrin		Deltamethrin	
	Before	After spiking	Before	After spiking	Before	After spiking	Before	After spiking	Before	After spiking	Before	After spiking	Before	After spiking
Batch 1	93 ±1	780 ±38	–	952 ±102	43 ±4	1 042 ±103	–	1 016 ±81	977 ±66	2 308 ±156	36 ±5	1 219 ±16	93 ±3	1 402 ±42
Batch 2	171 ±1	922 ±37	–	981 ±21	53 ±1	1 079 ±16	–	1 053 ±31	976 ±16	2 359 ±50	–	1 203 ±58	96 ±5	1 400 ±39
Batch 3	164 ±3	852 ±77	–	976 ±56	31 ±0	1 129 ±86	–	1 021 ±83	906 ±64	2 968 ±246	–	1 129 ±34	97 ±6	1 336 ±45
Batch 4	139 ±3	807 ±67	–	973 ±65	96 ±7	1 032 ±64	–	953 ±78	1 139 ±17	2 266 ±192	–	1 161 ±60	103 ±3	1 295 ±40
Batch 5	179 ±2	888 ±22	–	1 052 ±48	32 ±2	1 139 ±35	–	1 096 ±50	663 ±28	2 352 ±40	–	1 214 ±42	60 ±3	1 356 ±72
Batch 6	91 ±5	887 ±57	–	1 160 ±32	91 ±4	1 269 ±51	–	1 216 ±72	333 ±18	2 179 ±123	–	1 395 ±53	31 ±3	1 426 ±118
Batch 7	474 ±16	1 359 ±60	–	1 208 ±105	314 ±12	1 687 ±89	–	1 401 ±63	2 170 ±35	4 453 ±271	–	1 424 ±108	196 ±3	1 744 ±242
Batch 8	478 ±8	1 381 ±265	–	1 299 ±83	158 ±7	1 834 ±65	–	1 511 ±143	2 825 ±157	5 261 ±279	–	1 611 ±57	257 ±16	2 014 ±74
Batch 9	439 ±4	1 192 ±241	–	1 142 ±105	131 ±4	1 570 ±84	–	1 268 ±123	2 308 ±77	4 411 ±244	–	1 335 ±44	214 ±6	1 665 ±128
Batch 10	379 ±0	1 164 ±81	–	1 045 ±70	317 ±5	1 530 ±65	–	1 300 ±85	2 752 ±57	4 139 ±169	–	1 216 ±68	249 ±5	1 536 ±172
Average sample	231 ±0		13 ±1		109 ±4		6 ±1		1 410 ±22		– ±0		150 ±5	
Mean 10 samples	261 ±162	1 023 ±229	–	1 079 ±118	127 ±108	1 331 ±297	–	1 184 ±185	1 505 ±914	3 270 ±1 169	4	1 291 ±149	140 ±82	1 518 ±227
Theoretical dose		3 228		2 848		3 066		2 959		4 322		2 964		3 120
losses		68%		62%		57%		60%		24%		56%		51%

These poor observed recoveries were also reflected in the processing factors *i.e.*, the concentration ratio between germs and kernels (Table 9).

This result is not related to the hydrophobicity of the molecules, their molar mass, or their water solubility. Therefore, we hypothesized that pyrethroids and in a lesser extent, piperonyl butoxide were bound to the hydrophobic material of the grain cuticle. The cuticle in plants is coating sensitive organs like leaves and seeds to protect them against desiccation and excessive moisture. It is composed of polyesters of fatty acids and fatty alcohols, some waxes, and phenolic acids (Kunst and Samuels, 2003). Wax crystals at the surface could be sites of adsorption for of the pyrethroids. According to our hypothesis, organophosphates were more able to migrate toward the germs during the storage period while pyrethroids would remain bound to the cuticle (Wilde *et al.,* 2004).

To test this hypothesis, we carried out a new experiment in which a batch of new kernels was spiked with the same mixture of molecules according to the same protocol. The kernels were stored 60 days before being washed by solvents and analyzed for pesticides quantification.

The spiked kernels were then extracted following 3 different methods.

1 Direct analytical extraction of the molecules according to the original method (control);
2 10 min wash in hexane followed by analytical extraction (same method than 1);
3 10 min wash in acetonitrile (ACN) followed by analytical extraction (idem).

According to our hypothesis, if pyrethroids were adsorbed on the cuticle, and have not migrated on the kernels, the analysis after the solvents wash should find lower concentration than in the non-washed kernels. It was also expected that piperonyl butoxide would exhibit an intermediary behavior. Hexane being a solvent with strong hydrophobicity, it was also expected that this solvent would remove less efficiently the molecules that migrated in the kernels and that ACN will be more efficient for these molecules if the migration was just superficial.

The spiking was made on August 19th, 2020 by the same laboratory (SITONA AgroExpert) on 50 kg of corn (new batch supplied by a local retailer). The corn kernels were packed in plastic bags of approximately 17 kg stored at room temperature.

Table 11 shows the concentrations observed in the kernels according to the method of preliminary washing.

The pesticides remaining after ACN washing were above 100% for 3 organophosphates. This concentration effect could be explained by the fact that ACN extracted organic material from the outer layers of the kernels where these molecules were not present. Regarding the mutual variation of the extractability of the molecules and of processing factor, a clear similarity is appearing. Cuticle adsorbed molecules, those easy to remove by washing are those which have the lowest PF (Fig. 5). On the contrary, the molecules having migrated in the germs were not removed by the washes and have high processing factors.

Among organophosphates, fenitrothion may have a slightly lesser mobility and was still partially present at the periphery of the kernels. Piperonyl butoxide mobility in the corn kernels was intermediate and low in pyrethroids. ACN exhibited a lesser capacity of extraction than hexane regarding

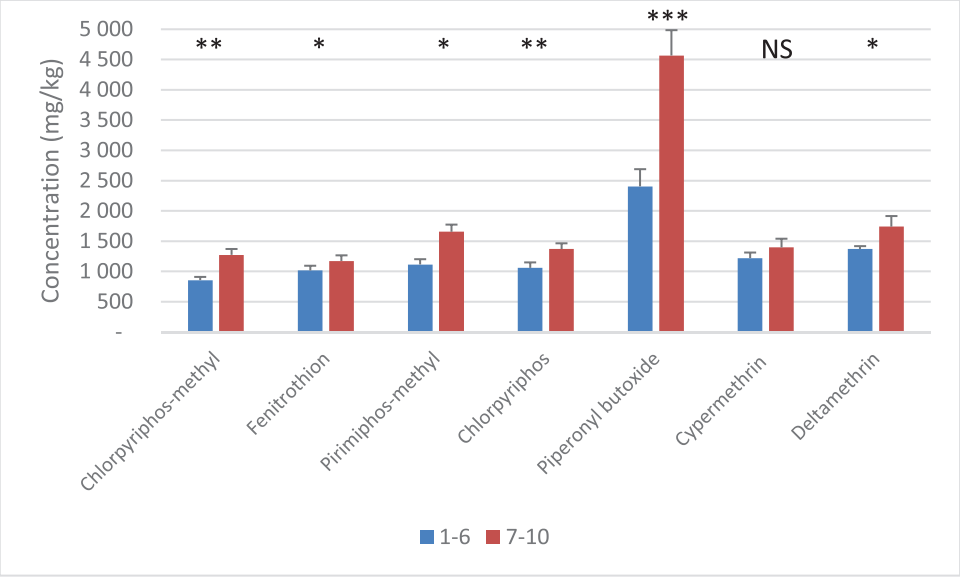

Figure 4. Average concentration after spiking for batches 1−6 *versus* 7−10, error bar indicating standard deviation of each group, t test probability: * $P(t) < 0.05$; ** $P(t) < 0.01$; ***$P(t) < 0.001$.

Table 9. Pesticides concentrations in the kernels ([K]) and in the germs ([G]) and processing factor ([G]/[K]).

Concentrations (µg/kg)	Maize kernels (mean ±sd)	Germs (mean ±sd)	Processing factor
Chlorpyriphos-methyl	1023 ± 88	12 920 ± 1 876	12.7
Fenitrothion	1079 ± 68	8 014 ± 1 896	7.4
Pirimiphos-methyl	1331 ± 67	13 797 ± 945	10.4
Chlorpyriphos	1184 ± 80	12 141 ± 1 120	10.2
Piperonyl butoxide	3270 ± 177	15 801 ± 1 126	4.8
Cypermethrin I	1291 ± 54	2231 ± 427	1.7
Deltamethrin	1518 ± 94	2091 ± 414	1.4

Table 10. Pesticide log $P_{o/w}$ values according to PubChem and assessment of quantitative recovery of the pesticides in the germs.

Molecule	Log $P_{o/w}$	Mass in grain (mg/100kg)	Mass in germs (mg/6.4 kg)	Transfer yield
Chlorpyriphos-methyl	4.3	102	83	81%
Fenitrothion	3.3	108	51	48%
Pirimiphos-methyl	4.2	133	88	66%
Chlorpyriphos	5.0	118	78	66%
Piperonyl butoxide	4.7	327	101	31%
Cypermethrin	6.6	129	14	11%
Deltamethrin	6.2	152	13	9%

Table 11. Concentration measured in the kernels after solvent wash and without solvent wash.

Concentrations in mg/kg	Control (1)	Hexane washed (2)	ACN washed (3)	Pesticide remaining after	
				hexane wash	ACN wash
Chlorpyriphos-methyl	3.03	2.14	3.28	71%	109%
Fenitrothion	3.06	2.29	2.86	75%	93%
Pirimiphos-methyl	3.11	2.11	3.20	68%	103%
Chlorpyriphos	3.14	2.28	3.44	73%	109%
Piperonyl butoxide	3.60	1.42	1.75	39%	48%
Cypermethrin I	3.90	0.52	0.81	13%	21%
Deltamethrin	4.36	0.42	0.76	10%	18%

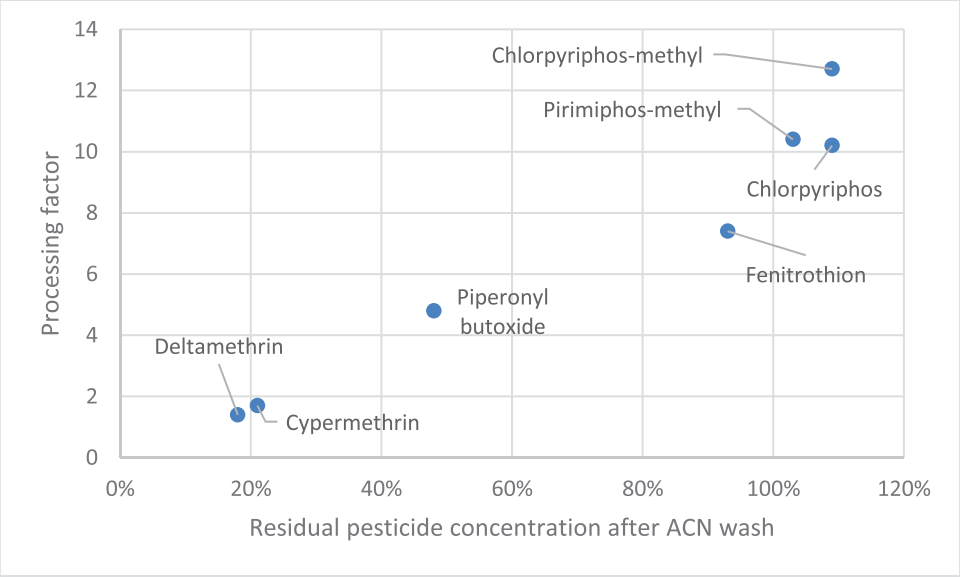

Figure 5. Comparison between the residual concentration of pesticides after a 10 minutes wash of the seeds in acetonitrile (ambient temperature) and processing factor (concentration in germs/concentration in kernels).

Table 12. Pesticides concentration in the germs, the oil extracted from the germs and the residual meals (batches 1, 2 & 3).

Material	Values in µg/kg	B1	B2	B3
Germs	Chlorpyriphos-methyl	10 974 ± 438	14 718± 147	13 069± 168
	Fenitrothion	6 693 ± 113	10 186± 155	7 162± 110
	Pirimiphos-methyl	12 711 ± 202	14 249± 269	14 431± 176
	Chlorpyriphos	11 316 ± 137	11 690± 141	13 416 ± 317
	Piperonyl butoxide	14 668 ± 46	15 815± 398	16 920 ± 66
	Cypermethrin	1 980 ± 121	1 989 ± 101	2 725 ± 149
	Deltamethrin	1 862 ± 4	1 841 ± 32	2 568 ± 122
Meals	Chlorpyriphos-methyl	41 ± 2	83 ± 10	23 ± 0
	Fenitrothion	25 ± 0	69 ± 2	31 ± 2
	Pirimiphos-methyl	401 ± 0	437 ± 6	212 ± 11
	Chlorpyriphos	552 ± 14	534 ± 26	395 ± 10
	Piperonyl butoxide	1 127 ± 37	1 133 ± 56	802 ± 0
	Cypermethrin	179 ± 2	203 ± 27	194 ± 2
	Deltamethrin	263 ± 13	284 ± 11	276 ± 9
Oils	Chlorpyriphos-methyl	22 019 ± 292	27 819 ± 617	24 739 ± 467
	Fenitrothion	12 206 ± 1237	17 391 ± 131	12 420 ± 204
	Pirimiphos-methyl	25 082 ± 2843	26 777 ± 48	26 563 ± 142
	Chlorpyriphos	23 124 ± 2660	22 295 ± 129	24 893 ± 157
	Piperonyl butoxide	29 099 ± 3257	32 187 ± 1168	32 701 ± 205
	Cypermethrin	5 041 ± 125	4 726 ± 94	5 454 ± 33
	Deltamethrin	4 293 ± 151	4 196 ± 214	4 556 ± 64

Table 13. Concentration of pesticides in kernels, germs, processing factors calculated from these concentrations and maximal residue levels.

	Kernels concentrations ([K]) (mg/kg)	Germs concentration ([G]) (mg/kg)	Processing factor ([G]/[K])	MLR in corn kernels (EU database, mg/kg)	Assessed limit in crude oil (mg/kg)
Chlorpyriphos-methyl	1.02	12.92	12.7	0.01	0.13
Fenitrothion	1.08	8.01	7.4	0.05	0.37
Pirimiphos-methyl	1.33	13.80	10.4	0.5	5.2
Chlorpyriphos	1.19	12.14	10.2	0.01	0.10
Piperonyl butoxide	3.27	15.80	4.8		
Cypermethrin	1.29	2.23	1.7	0.3	0.51
Deltamethrin	1.52	2.09	1.4	2.0	2.8

all the pesticides. The cuticle does not cover 100% of the corn kernel. The tip cap of the grain, *i.e.*, the part by which the kernels are attached to the cob are deprived of cuticle and therefore present a lesser ability to retain the pyrethroids. This explains that a significant percentage of these pyrethroids were able to reach the germs. These results are in accordance with studies related to milling (Mahugija *et al.*, 2017; JMPR, 2009; Cui *et al.*, 2022) which found higher pesticides concentrations in brans and germs.

In conclusion, the surprising results observed on the processing factors of pyrethroids can be explained by their capacity to remain adsorbed on the surface of the kernels or inside the layer of hydrophobic substance forming the cuticle.

3.2.3 Pesticide transfer from the germs to the oil and the defatted meal

The concentration values of Table 12 are confirming with good accuracy the assumption that hydrophobic pesticides are 100 % located in the oil in oleaginous materials. The average ratio of concentrations between oils and germs (processing factors) were 1.93, 1.76, 1.90 and 1.94 for chlorpyriphos-methyl, fenitrothion, pirimiphos-methyl and chlorpyriphos respectively. The lower ratio for fenitrothion is not explained by a lesser extraction of the meal because the residues of this organophosphate in the meal are very low (42 µg/kg).

The concentration ratio between oils and germs were 1.98, 2.31 and 2.12 for piperonyl butoxide, cypermethrin and deltamethrin. These values are higher than expected in pyrethroids. No clear reason can be advanced for explaining this discrepancy which does not question the big picture and the residues in the meal are low enough to confirm the general trend.

3.2.4 Processing factors from kernels to crude oil

Table 13 gives the overall processing factor allowing to assess the acceptable limit concentration in crude oils from maximum residue level given by the European regulation. Among the organophosphates, fenitrothion exhibit a lesser transfer to the germs when compared to other organophosphates. This difference cannot be explained by stability, chlorpyrifos-methyl being less stable than fenitrothion but possibly, by a lesser hydrophobicity (*cf.* Table 9). Piperonyl butoxide is relatively less transferred taking in account its initial concentration. More surprising was the low transfer of the pyrethroids. Considering their high degree of hydrophobicity, one would expect a strong migration toward the germ oil. As explained in paragraph 3.2.2, this weak transfer is mostly explained by the bounding of the pyrethroids to the kernels skin.

FEDIOL did not expect to have the same processing factors for maize, but some differences were surprisingly large. The pyrethroid group is the mostly affected by the starch barrier, but then from germ to oil this group has the highest processing factor. From this point of view, recovery of pesticide during oil extraction is following the same pattern than with oilseeds.

4 Conclusion

The general idea that emerges from this work is that we will have to be more cautious when talking about the migration of pesticides to the lipids contained by oilseeds, from what we have observed, not all molecules have the same capacity to migrate inside the seeds. When oilseeds are extracted directly without going through a prior fractionation step, this is of no practical consequence since the solvent used for extraction comes into contact with the pesticide remaining on the surface. As a result, the pesticide molecules, whether fixed to the seed coat or having migrated into the lipid fraction of the seeds, pass into the miscella and end up in the oil after distillation of the solvent. On the other hand, in the case of grains which, like maize, undergo a fractionation stage before the oil extraction, the molecules on the surface of the grain are no longer recoverable at the time of extraction. A similar phenomenon could occur in the case of oilseeds that undergo dehulling, such as sunflower. Hull of sunflower seeds are covered with wax which could fix pyrethroids. However, this could be mitigated by the wax transfer occurring during the sorting operations. Furthermore, this species does not have the starch barrier which in maize grains may have contributed to limiting the migration of pyrethroids.

The general proposition of FEDIOL to consider that hydrophobic pesticides are very likely to migrate in the oil of the oleaginous material remains broadly relevant at the end of this study in regard to the processing factor observed from germs to crude oil. Unfortunately, the MRL for pesticides are

only given for the grains. If the theory was verified, the average processing factor should have been close to inverse of oil concentration (1/0.037 = 27). Here, the highest processing factor was found for chlorpyriphos-methyl with a value of 12.7. In consequence, lower residues in crude oil will have to be tolerated than by use of the FEDIOL proposition.

Authors' contributions

Patrick Carré: Conceptualization, Investigation, Writing; Florence Lacoste: Conceptualization, Methodology, Project Administration, Validation; Loïc Leitner: Investigations, Data Curation, Supervision; Jean-Noël Arnaud: Investigation, Julie Roiz: Conceptualization, Methodology.

Conflicts of 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.

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