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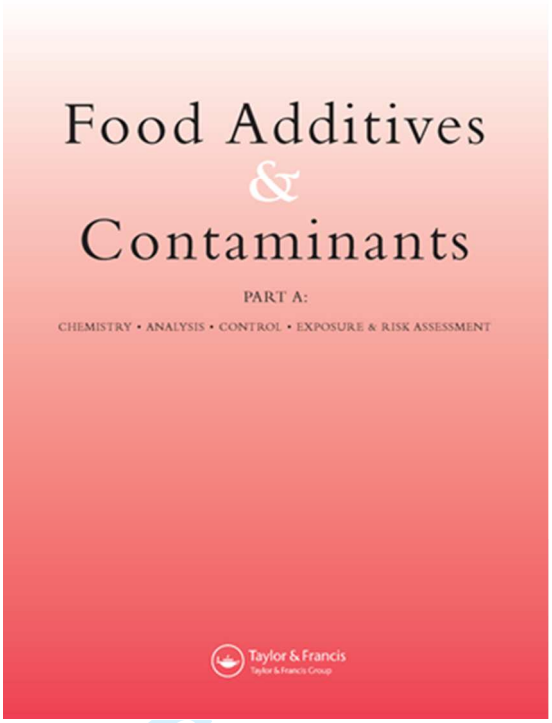
Published in:
Food Additives & Contaminants: Part A - Chemistry, Analysis, Control, Exposure & Risk Assessment

Link to article, DOI:
10.1080/19440049.2017.1339915

Publication date:
2017

Document Version
Peer reviewed version



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Journal:	*Food Additives and Contaminants*
Manuscript ID	TFAC-2017-099
Manuscript Type:	Original Article
Date Submitted by the Author:	24-Feb-2017
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Methods/Techniques:	Chromatography - GC/MS, Chromatography - LC/MS
Additives/Contaminants:	Pesticides
Food Types:	Cereals
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ABSTRACT

This study investigated the effects of particle size and milling temperature on the extraction efficiencies of pesticide residues from cereal flour. Samples of cereal grains were milled using a centrifugal mill with four different sieves (0.2, 1.0, 3.0 and 5.0 mm) and a knife mill both at room temperature and after freezing at -80 ºC overnight. The incurred pesticides in the test materials were extracted by the QuEChERS method and analysed by LC-MS/MS and GC-MS/MS. The particle size distribution for the milled samples was determined using a vibratory sieve shaker. The results confirmed that smaller average particle sizes increase the extraction efficiency up to 31%, with all other factors held constant. The cereals milled at room temperature produced lower pesticide recoveries compared to cereals milled when still frozen, especially for heat-sensitive pesticides. Furthermore, milling frozen grains was easier and resulted in more homogeneous samples with smaller relative particle sizes (RPS).

KEYWORDS: Pesticides, QuEChERS, extraction efficiency, cereals, milling, particle size

Introduction

Since ancient times, cereals have been a large component of the human diet. Because cereals are one of the foods most produced and consumed in the world, their safety is important. Cereals frequently receive intense applications of pesticides over their whole growing and post-harvesting periods. Therefore, food-safety issues introduced by food contamination from pesticide residues are becoming increasingly important. To ensure a high level of food safety, several techniques for pesticides residue analysis have been developed. Among the different analytical approaches developed so far, the QuEhERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method has become particularly important in this field due to its inherent advantages (Anastassiades et al. 2003; Lehotay et al. 2005; AOAC 2007; Sack et al. 2011). Several factors may have an impact on pesticide extractability from cereals, such as the physicochemical properties of the pesticides, the accumulation of pesticides in the inner or cuticle parts of the grains, the time of pesticide application, and the crop type (Hepperle et al. 2015). The incurred pesticide residues are not always easy to extract because they might be enclosed in cells, starch, or fat particles or may undergo strong interactions with the matrix. Factors such as the extraction temperature and time, the choice of solvent, the sample to solvent ratio, and the sample particle size can greatly influence the extraction efficiency of certain pesticides. It is common to study the role of these factors and optimise them when developing extraction methods, with the exception of the role of the sample particle size. However, the effects that homogenisation/grinding to different extents or by different procedures have on the extractability of analytes are not commonly studied or published. In Anastassiades (Anastassiades et al. 2003), the authors describe the developmental work behind the QuEChERS method and state that laboratories rarely evaluate the quality of the sample comminution step in their quality control procedures and that the procedures used vary significantly from laboratory to laboratory. Common methods

include homogenising/grinding or milling the sample using a standard procedure or to the smallest particles possible with the equipment available. This strategy is based on the assumption that a smaller particle size of the sample results in more efficient extraction because the surface area that is accessible to the solvent increases with decreasing particle size.

To our knowledge, literature highlighting the effect of particle size on the extraction efficiency of pesticides in flour is very scarce. Recently, Hepperle et al. (Hepperle et al. 2015) studied milled and re-milled rice and wheat. However, except for chlorpyrifos in a wheat sample, they did not find higher extraction yields for other pesticides as a result of a higher comminution grade. Some data are available for other matrices. A relationship between smaller particle sizes and higher extraction efficiencies has been reported for the extraction of organochlorine pesticides from ginseng root (Quan et al. 2004); although, it has also been reported that the extraction efficiency of organochlorine pesticides from homogenised sunflower seeds is lower with a particle size of 0.2 mm than with a particle size of 0.8 mm because the sample with the low particle size becomes compact (R. Prados-Rosales, J. Luque García 2003). Thus, there may be a lower limit for which the positive relationship between the particles size and the extraction efficiency no longer exists. In other fields of research, studies have also confirmed the expected higher extraction efficiency with lower particle size, e.g., for the extraction of paprika by supercritical fluid extraction (Nagy & Simándi 2008) and for the extraction of natural constituents (berberine and aristolochic acids) from medicinal plants by pressurised liquid extraction (PLE) (Ong et al. 2000). Particle size was thought to be less critical for the extraction efficiency when using PLE because of the high pressure applied, but the state of the sample is also of importance when using this technique.

Therefore, thorough homogenisation to obtain small and homogeneous particle sizes is expected to be of great importance in the analysis of pesticide residue in cereals and all other

fields of quantitative analysis. Nevertheless, sample particle size is not evaluated when laboratories participate in proficiency tests regarding pesticide residue analysis. The proficiency test materials are homogenised/grinded/milled by the provider of the test. For a European reference laboratory, it is a priority to provide knowledge-based advice to optimise and to harmonise the analytical performances of the European laboratories involved in pesticide residue control. The aim of the present study was therefore to study whether there is a relationship between the particle sizes of wheat, rye, oat and barley flour and the efficiency with which the incurred pesticides are extracted. Additionally, the heat produced during milling may affect the heat sensitive pesticides. Therefore, we investigated whether freezing the cereal grains before milling could prevent pesticide losses.

Experimental

Chemicals

The pesticide standards (all with a purity > 96%) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Acetonitrile (HPLC Grade S) was purchased from Rathburn Chemicals (Walkerburn, UK) and acetic acid and ammonium acetate were from Merck (Darmstadt, Germany). The magnesium sulphate was purchased from J.T. Baker, Aventor Performance Materials B.V (Center Valley, PA 18034, USA), sodium chloride from Merck & Co. (Whitehouse Station, NJ, USA), sodium citrate dehydrate and sodium citrate sesquihydrate from Sigma Aldrich Chemie GmbH (Taufkirchen, Germany), and the clean/up sorbents PSA from Agilent Technologies (Santa Clara, CA 95051, USA) and C18 from International Sorbent Technology Ltd. (Gengoed Mid Glam, UK). The pesticide standard stock solutions of 1 mg/ml were prepared in toluene and stored at −18 °C in ampoules with an argon atmosphere. A standard mixture of 10 µg/ml in acetonitrile was prepared from these

stock solutions. Working solutions were prepared by diluting this standard mixture and finally matching them 1:1 with the extracts of blank flours (not containing pesticide residues) to obtain a concentration range of 0.0003–0.333 µg/ml. The extracts used for the matrix matching were obtained by the extraction and clean-up procedure described in Section 2.2, which was used as our standard procedure.

Sample and sample preparation

The samples used in this study were cereal grains grown and sprayed in the field produced in connection with the production of proficiency test material for the four EUPTs: EU-PT-C2 (wheat), C3 (oat), C4 (rye) and C6 (barley). The levels of the incurred pesticides in the test material, which are determined with our in-house method involving milling with a 1 mm sieve size, extraction by the in-house QuEChERS method (see section 0) and analysis by LC-MS/MS and GC-MS/MS, are presented in Table 1.

Samples of the cereal grains along with the corresponding blanks were milled at room temperature using a centrifugal mill (Retsch, Haan, Germany) with four different sieves: 0.2, 1.0, 3.0 and 5.0 mm and a coffee grinder (Bodum, Switzerland). To study the effects of temperature, the oat and rye samples were also milled after freezing at -80 ºC overnight. Milling of the test materials was performed in duplicate, and the untreated samples (blank) were treated individually. For each milling, 200 g of sample was employed.

The water content of the grain was determined by drying 5 g (in duplicate) under a vacuum at 70°C for at least 16 hours or until the gravimetric determination stabilised.

Particle size distribution analysis

The particle size distribution from the milled samples of wheat, oat, rye and barley was determined using a vibratory sieve shaker (Retsch AS 200, Haan, Germany). The sieving parameters (sample size, sieve time and amplitude) were optimised using different sample sizes (50 and 100 g), amplitudes (2-3 mm) and sieving times (10-20 min). The optimal sieving conditions that produced the most efficient separation of the flour particles were using 50 g of flour samples with 20 min of sieving time at amplitude of 2.5 mm. The flour samples were separated into fractions according to the sieve mesh sizes ranging from 50 μm to 4.8 mm. Sieving of the test materials was performed in duplicate.

The relative particle size (RPS) of each milled sample was calculated using the following formula:

$$RPS = \sum_{i=1}^{n} (AS * SA)$$

RPS: relative particle size

AS: average sieve mesh size (calculated as the average between sieve n and sieve n-1)

SA: sample amount in sieve n (g)

The size distributions and particle shapes of the selected samples were also observed under a Leica (DMR) light microscope. The microscope was attached to a camera (Leica DFC295) to capture the digital images, and image analysis software (Image-Pro Plus 7.0; Media Cybernetics, Bethesda, MD, USA) was used for the size measurements.

Pesticides extraction and clean-up procedure

The samples were extracted using our in-house standard procedure for cereals (in accordance with QuEChERS EN 15662) (Herrmann & Poulsen 2015). Briefly, 5.0 g of milled cereal was added to 10 ml of cold water and immediately extracted with 10 ml of acetonitrile by shaking the tube for 1 min by hand. To aid the extraction, a ceramic homogeniser was included. Next, 4.0 g of magnesium sulphate, 1.0 g of sodium chloride, 1.0 g of sodium citrate dihydrate and 0.5 g of sodium citrate sesquihydrate were added. After 1 min of shaking by hand followed by centrifugation for 10 min at 4300 G, 8 ml of the supernatant was transferred to a clean tube and stored at −80 °C for minimum 1 h. The extracts were then thawed, and when they were still very cold, they were centrifuged at 4300 G for 5 min. Thereafter, 6 ml of the cold supernatant was mixed with 150 mg of PSA and 900 mg of magnesium sulphate. After shaking for 30 s and centrifuging for 5 min at 3400 G, the extract was added to 40 µl of 5% formic acid solution and analysed by GC-MS/MS and LC-MS/MS.

Chromatographic separation and detection

GC-MS/MS analysis was performed on a Quattro Micro Tandem GC–MS/MS (Waters, USA). The system consisted of a PAL-GC Auto sampler, an Agilent GC 6890N and a Quattro Micro Tandem mass spectrometer. The GC was equipped with a Gerstel PTV injector for large volumes, and 4 µl of sample was injected. The injector program started with an initial temperature of 30 ºC for 0.8 min followed by a ramp of 480 ºC/min to 290 ºC. The temperature was held for 2 min and was then raised at a rate of 720 ºC/min to 330 ºC to clean the injector. The GC oven program started with an initial temperature of 60 ºC held for 3 min, followed by a ramp of 30 ºC/min to 180 ºC. This temperature was held for 0.8 min, before being increase at a rate of 5 ºC/min to 280 ºC and subsequently held for 3 min. To

clean the column, the temperature was raised at a rate of 40 ºC/min to 300 ºC for 10 min and 120 ºC/min to 310 ºC for 1 min. The chromatographic separation was performed on a RESTEK, Rxi®-5 ms, 30 m, 0.25 mm ID, 0.25 µm df column with a constant flow of 1.3 ml/min of helium as the carrier gas. The temperatures of the transfer line and ion source were set at 250 ºC and 180 ºC, respectively. The mass spectrometer was operated in electronic ionisation mode (EI, 70 eV). The analysis in scan mode was employed to obtain TIC chromatograms for the determination of the intensities of the matrix peaks. MRM was used to perform the mass spectrometric quantification of the pesticides. The employed MRM transitions, retention times and collision energies are listed in Table 2.

The LC-MS/MS analysis was performed on an HP1100 liquid chromatograph (Agilent Technologies, Palo Alto, CA, USA) connected to a Micromass Quattro Ultima Triple Quadrupole Instrument. The injection volume was 10 µl. The chromatographic separation was performed on a Genesis C18 column, 100 mm × 3 mm, 4 m pore size, (Gracevydac, Hengoed, UK). Before the separation column was a Phenomenex SecurityGuard column, C18 ODS, 4 mm × 2 mm (Cheshire, UK). Solvent A was an ammonium acetate/acetic acid solution containing 20 mM of each. Solvent B was 95% methanol and 20 mM each of ammonium acetate and acetic acid. The total flow rate of eluents A and B was 0.3 ml/min. The initial gradient was 100% A, decreasing to 50% A after 2 min and 0% A after 20 min. Solvent A was held at 0% until 24 min. The total run time was 30 min. The retention times are shown in Table 2. Ionisation was performed using electrospraying in positive ion mode (ESI+) and the mass spectrometer in MRM mode. The capillary voltage was set to 1.0 kV. The source temperature was 120 ºC, and the desolvation temperature was 350 ºC. Nitrogen was used as the desolvation gas (flow 550 l/h) and cone gas (flow 50 l/h), and argon was used as the collision gas at a pressure of 1.7 × 10−3 mbar. The MRM transitions, retention times and collision energies employed for the LC-MS/MS analysis are listed in Table 3.

The quantification was based on the bracketing calibration curves of five matrix matched standard solutions, covering the relevant concentration range. Exact matrix matching was employed, i.e., the pesticide content of the oats test material was quantified using the calibration solutions matrix-matched with the oats blank material, and for rye, using the calibration solution matrix-matched with the rye blank material.

Results and discussion

To elucidate the often-overlooked effect of the particle size on the extraction efficiency, we have studied the possible relation between the decreasing particle size and the efficiency of 33 incurred pesticide residues extracted from cereal flours, i.e., wheat, oat, rye and barley.

Particle size distribution of flour

To find justification for any variations in the pesticide extraction efficiencies from grain in relation to the particle size, we investigated the particle size distributions at each of the milling sizes. Milling with sieve sizes of 1.0 mm and 3.0 mm gave more or less an even particle size distribution. However, we observed that milling with a sieve size of 0.2 mm apparently results in a large proportion of particles larger than 0.2 mm. For instance, in oats and rye milled with a sieve size of 0.2 mm, more than 65% of the particles were larger than 0.2 mm (Figure 1b & 1c). Milling at a sieve size of 5.0 mm and with the knife mill resulted in a very in-homogeneous flour, containing both small (<0.05 mm) and large particles (>0.7 mm) (Figure 1a-1d). The average particle size of all the cereals showed that the knife mill produces greater amounts of large particles (more than 50% of the particles were >0.7 mm). The size distribution showed that grinding the frozen samples resulted in more homogeneous

flour samples with greater proportions of smaller particles (Figure 1e & 1f). Cryogenic milling resulted in greater amounts of smaller particles, especially at a mill size of 0.2. However, cryogenic milling did not show such effects on particle size of the flours milled with a sieve size of 5.0 mm and the knife mill.

As expected, the relative particle size (RPS) was smaller for grains milled with a smaller sieve size. However, the RPS of oat flour at each sieve size (except for the sieve size of 3.0 mm) was the largest among the four grains studied; whereas, wheat flour showed the smallest RPS among the four flours. These differences may be due to differences in the hardness, moisture and fat content of the grains. Cryogenic milling resulted in a smaller RPS for each sieve size, except for the sieve size of 0.2 mm and the knife mill, which did not exhibit any considerable differences. The knife mill resulted in the largest RPS of the tested milling conditions.

Inspection of the shape of the flour particles under a microscope revealed that the milled cereals have very inhomogeneous particles from round to long shapes (Figure 2). From the dimensions of the particles, it can be concluded that the particles that are smaller in just one dimension than the mesh size of the sieve can pass through it. Consequently, some of the particles from the flour milled with a specific mesh size of the sieve are found to be larger than the sieve size when sieved using a vibratory sieve shaker.

Extraction efficiency of different particle sizes.

The recoveries of the incurred pesticides from the flours milled with the different sieve sizes were calculated for each type of grain and compared with the recoveries obtained for the corresponding samples milled with a sieve size of 1.0 mm. A comparison of the average recoveries, with all of the pesticides summed, showed that the extraction efficiencies

improved by up to 31% when the sieve mesh size was reduced from 5.0 mm to 0.2 mm (Figure 3). The average recoveries for all cereals were 116, 100, 97, 85, and 80% when milled with a sieve size of 0.2, 1.0, 3.0, 5.0, and the knife mill, respectively. The average recoveries, with all of the pesticides summed, ranged between 92-106% for wheat, 76-120% for oat, 74-105% for rye, and 87-132% for barley when milled with sieve sizes of 0.2-5.0 mm.

For all four types of cereals, a similar pattern was observed; the highest extraction efficiencies were achieved when the samples where milled with a sieve size of 0.2 mm, except for some of the detected pesticides in wheat (Figure 4). The relationship was most clearly demonstrated by the results obtained for oats and rye (Figure 4c and 4d, respectively). The extraction efficiency for grain milled using the knife mill varied for the different types of cereals and pesticides (Figure 4c-d). Oats milled with the knife mill showed the lowest extraction efficiency for all of the pesticides, which is in good agreement with the fact that the knife mill provided the largest RPS. Rye milled with the knife mill showed the highest extraction efficiencies for azoxystrobin and carbendazim. It also exhibited slightly higher extraction efficiencies than the sieve size of 5.0 mm for most of the pesticides. This finding could be due to the very inhomogeneous flour produced by the knife mill, which contained both very small and very large particles. As mentioned in the previous section, the amount of particles with smaller sizes (0.2 mm) was higher compared to milling at 5.0 mm. In this study, all of the pesticides had been applied to the field 20-30 days before harvesting, except for malathion and chlorpyrifos-methyl, which were applied 10 days before harvesting. Therefore, it is expected that most of the malathion and chlorpyrifos-methyl residues remained in the outer parts of the grain.

It is observed that the extraction efficiency of pesticides was less affected by the particle size for wheat than for the other grains. This could be related to the fact that in oats and barley, the husk is fused together with the bran, while wheat and rye are naked cereals (P. Koehler

2013). When the grain matures (dries) in the field, the water evaporates from the oats/barley hull; whereas for rye/wheat, it evaporates from the bran/surface of the grain. It would therefore be expected that the residues found in wheat are, to a large extent, associated with the bran of the grain; whereas, residues in the oats remain in the grain. It is also assumed that pesticide residues are more evenly distributed in the oats/barley grains because if the residues have become more concentrated as a result of the drying process, it has occurred on the surface of the hull, which is removed before milling and analysis. However, the results obtained for rye in the current study did not fully explain this theory, and there might be other factors affecting the distribution of pesticides in rye.

During milling, and especially when milling with a sieve size of 0.2 mm and using the knife mill, heat was produced. This heat could result in the evaporation of water from the sample material. The loss of water could introduce an error in the study because the dry weight of the different millings would vary and in addition to a variation in the actual sample size extracted. Thus, an increase in the extraction efficiency for the samples milled to 0.2 mm could be the result of water evaporation and not from an increase in the accessibility of the finely milled samples. Therefore, the water content of the different flour samples was determined gravimetrically. A reduction in the water content was only observed for the samples milled to a particle size of 0.2 mm, i.e., 1.0-5.3%, and the greatest reduction was demonstrated for rye and barley. However, the correction of the quantitative results for the loss of water had little effect on the determined pesticide content and could therefore not explain the higher extraction efficiency observed when the grain was milled to a particle size of 0.2 mm.

Cryogenic milling

As explained above, the heat produced during milling was a result of the friction between the blades of the grinder. Thus, the lower recoveries of heat-sensitive pesticides could result during milling. Some of the pesticides, such as pyrethroids, which are represented by deltamethrin and lambda-cyhalothrin in the present study, have been reported as heat sensitive pesticides (Senneca et al. 2007). To study the effect of heat on the recoveries of the incurred pesticides, oat and rye grains were stored at -80 ºC overnight prior to milling. Figure 4e and 4f show that the recoveries of pesticides from frozen oats and rye are generally comparable with the recoveries found for samples milled at room temperature. For most of the pesticides, especially in the oat grains, a slightly higher recovery was observed for the samples milled at low temperatures. Thus, the present study showed that milling at a low temperature may improve the recoveries of some pesticides, but overall the results produced are similar. Although, milling at a low temperature made the milling process itself easier, especially for oats, by reducing the tendency of the flour to clot.

Carbendazim, chlorpyriphos-methyl, deltamethrin, lambda-cyhalothrin, malathion, and pirimiphos-methyl have been reported as thermally unstable compounds that undergo evaporation, degradation or polymerisation during heating (B.S. Joia, G.R.B. Webster 1985; Robert Mestres 1992; Yoshihiro HORI, Takao CHONAN 1992; Sharma et al. 2005; Uygun et al. 2005). In fact, for some of the mentioned compounds (carbendazim, lambda-cyhalothrin, and pirimiphos-methyl), slightly improved recoveries were observed in the present study when milling was performed using frozen grains instead of grains at room temperature. Performing the milling on frozen grains increased the extraction recoveries not only of heat sensitive pesticides but also strobilurin analogue (azoxystrobin and kresoxim-methyl) and organophosphorus pesticides (Chlorpyrifos-methyl, malathion and pirimiphos-methyl), especially at larger mill sizes. Thus, the slightly higher recoveries indicated for the samples milled at low temperature in the present study may not be related to less heat-induced

pesticide degradation, but to a larger proportion of small particles in the flour milled at low temperatures compared to flour milled at room temperature, as described above (section 3.1 and Figure 1e & 1f).

Grinding at a larger sieve size (5.0 mm) and with a knife mill resulted in very inhomogeneous flour with the largest relative particle size (RPS). Cryogenic milling resulted in more homogeneous flour samples with greater proportions of smaller particle sizes and smaller RPS. The present study supports the assumption that small particle sizes increase pesticide extraction efficiencies with all other factors remain equal. The observed effect of the particle size was demonstrated for the incurred pesticides and not the spiked pesticides. A comparison of the average recoveries, when all of the pesticides are summed, showed that the extraction efficiencies improved by up to 31% when the sieve mesh size was reduced from 5.0 mm to 0.2 mm. The extraction efficiency of the pesticides in wheat was influenced less by particle size than in the other grains. The extraction efficiency for grains milled using the knife mill varied for the different types of cereals and pesticides. In general, cereals milled at room temperature produced lower pesticide recoveries compared to low-temperature milling, especially for the heat-sensitive pesticides. Furthermore, when grinding at low temperatures, the milling process itself was easier, especially for oats, because of a reduction in the tendency of the flour to clot.

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Figure captions

Figure 1. Particle size (mm) distribution of a) wheat, b) barley, c) oats, d) rye milled at room temperature and e) oat, f) rye milled frozen with sieve size at 0.2, 1.0, 3.0, 5.0 mm and the knife mill.

Figure 2. A microscopic image of the different particle sizes from oats milled at a 2.0 mm sieve size.

Figure 3: The average recovery for all pesticide residues in all four cereals (wheat, oats, rye and barley) milled at room temperature with sieve sizes of 0.2, 1.0, 3.0, and 5.0 mm.

Figure 4: The recovery ((detected level/reference value)*100) of different pesticides in a) wheat, b) barley, c) oats, and d) rye milled at room temperature and e) oats and f) rye milled at a low temperature with sieve sizes of 0.2, 1.0, 3.0, and 5.0 mm and the knife mill (K.M.).

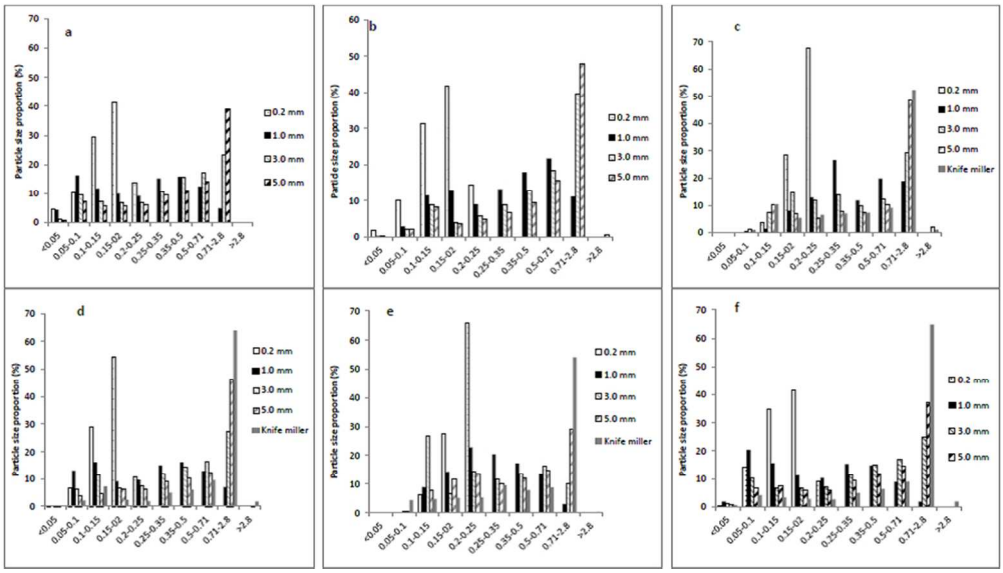

Figure 1. Particle size (mm) distribution of a) wheat, b) barley, c) oats, d) rye milled at room temperature and e) oat, f) rye milled frozen with sieve size at 0.2, 1.0, 3.0, 5.0 mm and the knife mill.

237x134mm (96 x 96 DPI)

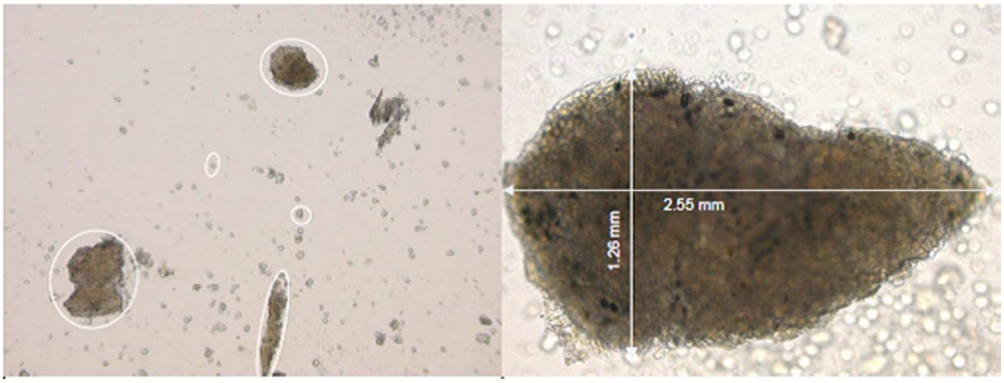

Figure 2. A microscopic image of the different particle sizes from oats milled at a 2.0 mm sieve size.

156x58mm (96 x 96 DPI)

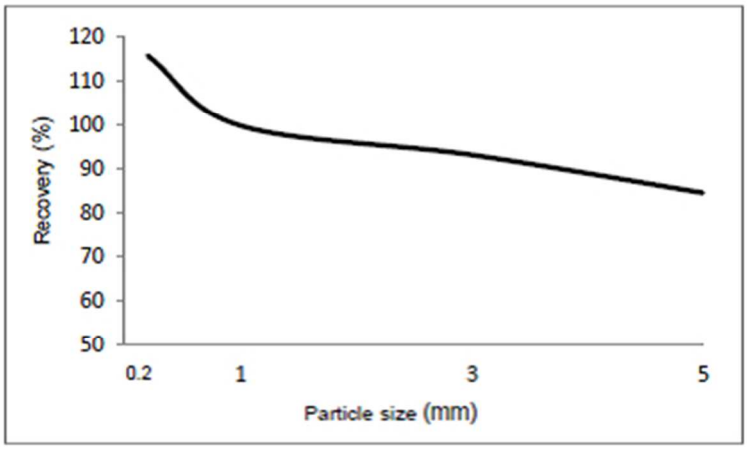

Figure 3: The average recovery for all pesticide residues in all four cereals (wheat, oats, rye and barley) milled at room temperature with sieve sizes of 0.2, 1.0, 3.0, and 5.0 mm.

99x59mm (96 x 96 DPI)

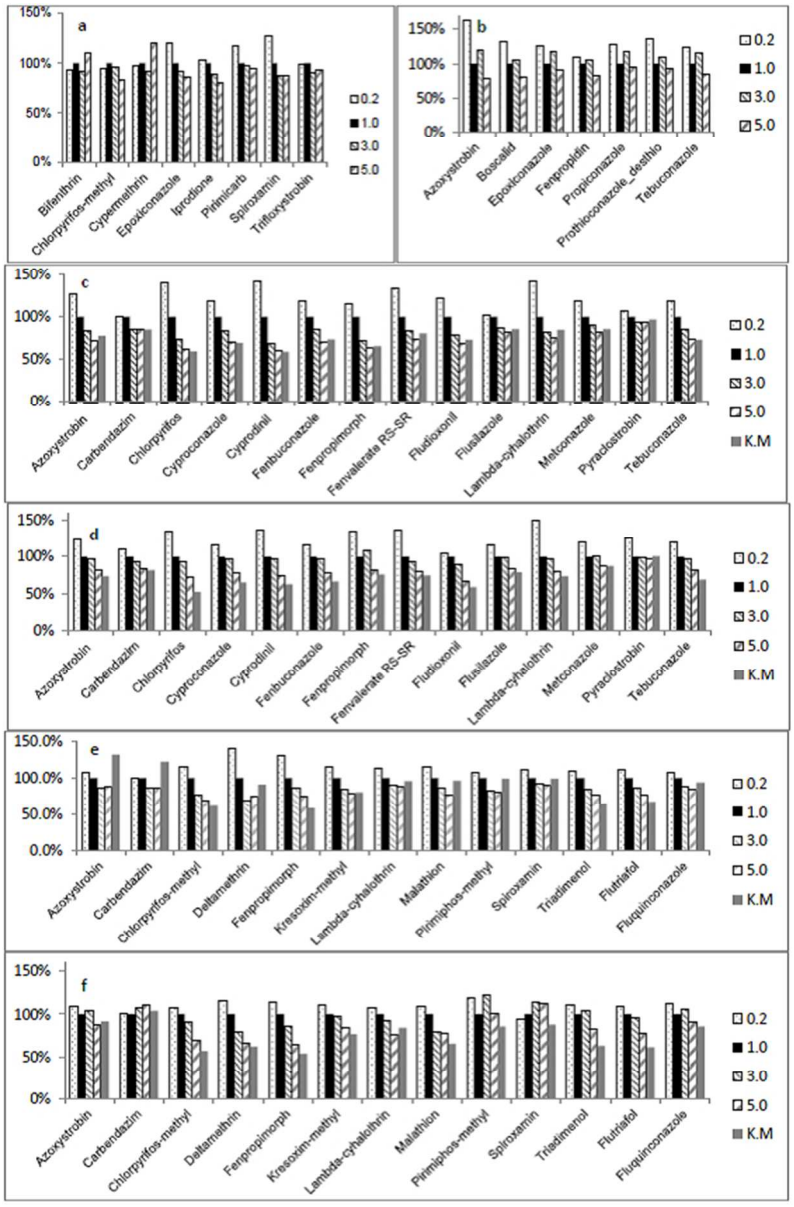

Figure 4: The recovery ((detected level/reference value)*100) of different pesticides in a) wheat, b) barley, c) oats, and d) rye milled at room temperature and e) oats and f) rye milled at a low temperature with sieve sizes of 0.2, 1.0, 3.0, and 5.0 mm and the knife mill (K.M.).

149x226mm (96 x 96 DPI)

Table 1. The levels of incurred pesticides (mg/kg) in grains milled at room temperature with a sieve size of 1.0 mm.

	Reference value (μg/g)			
Compounds	**Oats**	**Rye**	**Wheat**	**Barley**
Azoxystrobin	0.184	0.310	-	0.149
Bifenthrin	-	-	0.106	-
Boscalid	-	-	-	0.819
Carbendazim	0.440	1.121	-	-
Chlorpyrifos	1.054	-	-	-
Chlorpyrifos-methyl	-	0.094	0.151	-
Cypermethrin	-	-	0.1325	-
Cyproconazole	1.013	-	-	-
Cyprodinil	0.075	-	-	-
Deltamethrin	-	0.057	-	-
Epoxiconazole	-	-	0.181	0.569
Fenbuconazole	0.554	-	-	-
Fenpropidin	-	-	-	1.195
Fenpropimorph	0.162	2.063	-	-
Fenvalerate	0.099	-	-	-
Fludioxonil	0.108	-	-	-
Fluquinconazole	-	0.629	-	-
Flusilazole	0.814	-	-	-
Flutriafol	-	2.812	-	-
Iprodione	-	-	0.394	-
Kresoxim-methyl	-	0.425	-	-
Lambda-cyhalothrin	0.033	0.036	-	-
Malathion	-	0.094	-	-
Metconazole	0.564	-	-	-
Pirimicarb	-	-	0.032	-
Pirimiphos-methyl	-	0.048	-	-
Propiconazole	-	-	-	0.2175
Prothioconazole_desthio	-	-	-	0.080
Pyraclostrobin	0.816	-	-	0.001
Spiroxamin	-	1.15	0.059	-
Tebuconazole	1.787	-	-	0.513
Triadimenol	-	1.827	-	-
Trifloxystrobin	-	-	0.504	-

Table 2. The quantifier ions, qualifier transitions and collision energies for the GC-MS/MS amenable pesticides included in the test materials.

GC-MS/MS	Precursor ion-1	Product ion-1	Collision Energy 1 (V)	Precursor ion-2	Product ion-2	Collision Energy 2 (V)
Azoxystrobin	344	329	15	388	345	15
Bifenthrin	181	166	10	165	115	20
Boscalid	342	140	15	167	139	20
Carboxin	235	143	5	143	87	5
Chlorpropham	213	127	15	213	171	5
Chlorpyrifos	197	169	10	314	258	12
Chlorpyrifos-methyl	286	93	20	125	79	5
Cypermethrin	163	127	10	181	152	20
Cyproconazole	222	125	15	139	111	15
Cyprodinil	226	225	15	223	208	15
Deltamethrin-cis	181	152	10	253	174	10
Epoxiconazole	192	138	10	206	165	5
Fenbuconazole	198	129	10	129	102	15
Fenpropidin	98	70	10	99	71	10
Fenpropimorph	303	128	5	117	115	10
Fenvalerate	167	125	10	125	99	10
Iprodione	314	245	10	216	187	5
Isoprothiolane	290	118	10	290	204	2
Kresoxim-methyl	206	116	4	206	131	10
Lambda-cyhalothrin	197	141	10	208	181	10
Malathion	173	99	10	173	127	5
Metconazole	125	89	10	127	89	10
Pendimethalin	281	252	5	252	162	5
Pirimicarb	238	166	10	166	96	10
Pirimiphos-methyl	305	290	10	290	233	10
Propiconazole	173	145	15	259	173	15
Tebuconazole	250	125	15	125	89	10
Triadimenol	168	70	5	128	100	10
Trifloxystrobin	222	190	5	186	145	10

Table 3. The quantifier ions, qualifier transitions and collision energies for the LC-MS/MS amenable pesticides included in the test materials.

LC-MS/MS ESI+	Precursor ion-1	Product ion-1	Collision Energy 1 (V)	Precursor ion-2	Product ion-2	Collision Energy 2 (V)
Boscalid	343.1	307	20	343.1	140	25
Carbendazim	192	160	20	192	30	29
Cyprodinil	226	93	33	226	77	40
Epoxiconazol	330.11	121	23	330.11	91	41
Fludioxanil	247	180	27	247	126	33
Flusilazole	316.17	247	17	316.17	165	20
Isoprothiolane	291	231	10	291	189	21
Kresoxim-methyl	314	116	30	314	131	20
Pendimethalin	282.12	212	10	282.12	194	10
Pirimicarb	239	72	16	239	182	14
Pirimiphos-methyl	306	164	20	306	108	20
Prothioconazole_desthio	312	70	35	314	127	35
Pyraclostrobin	388.15	194	11	388.15	163	25
Spiroxamin	298.26	144	20	298.26	100	30