Effects of processing whole oats on the analysis and fate of mycotoxins and ergosterol

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Abstract

Grinding and dividing equipment were evaluated for their ability to comminute and divide ground oats in preparation for mycotoxin analysis. Four different grinders, using various settings, were evaluated for their ability to comminute oats and produce small particle sizes. Rotor beater type grinders produced the more desirable finer ground samples as compared to burr type mills. Four different division methods (manual scooping, rotary sample division, and two designs of gravity-fed dividers) were assessed for their ability to produce sub-samples with consistent particle size fraction distributions. No practical differences were observed on the particle size fraction distribution of test portions of finely ground oats produced using the four different division methods; therefore, no effects on mycotoxin analysis were anticipated. The effects of processing naturally contaminated whole oats on mycotoxin concentrations was also assessed. Laboratory scale dehulling, steaming, and kilning were examined. Dehulling showed the greatest impact and removed 60-100% of various *Fusarium*- and *Alternaria*-produced mycotoxins, as well as ergosterol, present on the naturally contaminated whole oats. Different from the other analytes studied, only 48% of the mycotoxin plant transformation product deoxynivalenol-3-glucoside was present in hulls and removed during dehulling. Steaming and kilning appeared to increase ergosterol in groats, as well as decrease deoxynivalenol and deoxynivalenol-3-glucoside. The observed inconsistent changes in concentrations of tentoxin after heat treatment of groats appeared to be due to sample heterogeneity.

Keywords: sampling, grinding, dehulling, heat, deoxynivalenol, ochratoxin A, deoxynivalenol-3-glucoside

1. Introduction

Oats (*Avena sativa*) are grown for food and feed uses. The three main markets for oats are milling, feed for livestock, and performance feed for horses (Bouphasiri and Verchomin, 2005). Due to the presence of an outer hull on the oat kernel, and relatively high lipid content compared to other cereals, various steps are required to process raw whole oats (i.e. oats with hull and other kernel components still intact and together) into consumable food and feed products. These steps can include physically removing the outer hull to produce groats, heat treatment to inactivate lipase enzymes and prevent rancidity, and flaking or milling. The specific processes used is guided by the ultimate use of the oats as food or feed. A good introduction to the various

processes involved in oat milling and processing for use in food products is presented by Scudamore *et al.* (2007).

As a cereal, oats are susceptible to fungal infection in both their growing and storage environments. Fungal infection can result in the accumulation of mycotoxins in the kernel, which leads to the exposure of food and feed consumers to these potentially hazardous compounds. Many studies have reported on the occurrence of mycotoxins in Canadian oats, and some have noted that the frequency and level of occurrence in oats is lower than for other cereal grains (Campbell *et al.*, 2002; Martos *et al.*, 2010). The *Fusarium*-produced deoxynivalenol (DON), HT-2 and T-2 toxins, as well as *Penicillium avenaceum*-produced ochratoxin A (OTA) have been measured in Canadian oats (Clear *et al.*, 2000; Tamburic-Ilincic, 2010; Tittlemier *et al.*, 2020;

Whitaker *et al.*, 2015a; Yan *et al.*, 2017). The less studied *Fusarium*-produced culmorin, enniatins, and beauvericin, as well as *Alternaria* mycotoxins, have also been observed in Canadian oats (Tittlemier *et al.*, 2020).

Due to the potentially heterogeneous nature of mycotoxins in cereal grains, analysis of grains for mycotoxins can be challenging. This is particularly the case for the analysis of OTA (Tittlemier *et al.*, 2011), and other mycotoxins that are associated with point sources in bulk grain, such as ergot alkaloids. As awareness of the importance of proper sampling and sample preparation is increasing, only a small number of studies have investigated the effects of sample preparation processes on the analysis of mycotoxins in grains (Grusie *et al.*, 2017; Tittlemier *et al.*, 2012). These studies examined the effect of sample size on variability of results, the distribution of mycotoxins amongst various particle sizes of comminuted grain, and the effect of particle size distribution of comminuted grain on measured concentrations. Neither of these studies included oats as a matrix.

Other studies have examined the fate of mycotoxins in oats undergoing various processing. Scudamore *et al.* (2007) studied the fate of DON, HT-2, and T-2 and other *Fusarium*-produced mycotoxins and derivatives in oats processed in a commercial mill in the United Kingdom. Ivanova *et al.* (2016) studied the fate of a wide variety of mycotoxins in Norwegian oats subjected to removal of the outer hull and subsequent abrasion of outer layers of the remaining groats. The effects of extrusion cooking on OTA (Lee *et al.*, 2017) and HT-2 and T-2 (Schmidt *et al.*, 2017), as well as the effects of making biscuits and muesli have also been studied (Kuchenbuch *et al.*, 2018). In general, significant decreases in mycotoxin contents were observed after dehulling, with some decreases after processing involving high heat as well. The degree of change in concentration varied with mycotoxin identity, as well as the specific processing conditions used. These have all focused on the fate of mycotoxins as oats are processed for food, and did not touch upon processing of oat samples for laboratory analysis.

There were four main objectives of the current research. The first was to compare the performance of different equipment when grinding whole oats by assessing the particle size distributions produced. The second was to compare the performance of different equipment when dividing ground oats by assessing the consistency of the particle size distribution produced. The third objective was to determine if mycotoxins and the fungal biomarker ergosterol (Seitz *et al.*, 1977) are associated with particular particle size fractions in order to help guide sampling and processing procedures to ensure representative samples are prepared for analysis. The final objective was to investigate if dehulling and heat treatment of oats affects mycotoxin

and ergosterol concentrations. This final objective will determine how mycotoxin concentrations are affected by processing and will help regulators develop accurate exposure assessments for consumers of oats.

2. Materials and methods

Comparison of grinders for the comminution of whole oats

The ability of four different grinders to comminute whole oats (i.e. oats with intact hulls) was assessed. The four grinders used were (1) a commercial coffee grinder (model KR 804, Ditting Maschinen AG, Bachenbülach, Switzerland); (2) the Romer Series II sub-sampling mill (Romer Labs, Union, MO, USA); (3) a Retsch SR 300 rotor beater mill coupled with a Retsch DR 100 vibratory feeder (Retsch, Haan, Germany); and (4) a Perten 3100 hammer mill (Perten instruments, Hägersten, Sweden).

Nine scenarios were used to grind 1 kg of whole oats (Table 1); oats used were representative of the quality exported for further processing. The scenarios incorporated different settings on the four grinders and were used to obtain various particle size distributions. The time taken to grind the oats and clean the grinder (including disassembly if required) to prepare for grinding a subsequent sample was recorded for each scenario by the laboratory personnel.

Analysis of particle size distribution of ground oats

Ground oats were sub-sampled using a rotary sample divider (Materials Sampling Solutions, Southport, Australia) with vibratory feeder (HI-VI models 15A and D20N; Eriez Manufacturing Co., Erie, PA, USA) to obtain a 100 g test portion of ground oats. The vibratory feeder amplitude was set to 50% and the carousel containing the 10 collection containers rotated at a constant speed of 12 rpm.

Table 1. Comparison of grinders and settings when grinding 1 kg of whole oats.

Grinder	Setting ¹	Time (min)
Ditting	coarse ('French Press')	5
Ditting	fine ('Turkish grind')	7
Romer	coarse (dial setting '1')	31
Romer	fine (dial setting '5')	37
Retsch	1000 µm	5
Retsch	750 µm	5
Retsch	500 µm	6
Perten	800 µm	31
Perten	500 µm	37

¹ Values refer to the nominal size of the openings on the sieve used in each scenario.

The particle size distribution of the ground oats produced by each grinding scenario was determined using a laboratory-scale plansifter. The particle size distribution was measured by mechanically sifting (at 220 rpm) the test portion through a rotary plansifter comprised of a series of vertically stacked sieves (0.33×0.33 m) with nylon mesh screens of various sized openings (1,180 µm, 475 µm, 300 µm, 180 µm, and 110 µm). After 10 s of sifting, the material remaining on top of the 1180 µm was collected. Material remaining on top of each of the four remaining sieves was collected after successive 5 s periods of sifting. Material smaller than 110 µm was collected from the bottom pan. All sifting was performed with no tapping of the sieves in order to maximise consistency of analysis amongst samples. Losses were calculated as the difference between the initial test portion mass and the total mass of collected fractions. Six particle size fractions were collected: >1,180 µm, >475 to 1,180 µm, >300 to 475 µm, >180 to 300 µm, >110 to 180 µm, and <110 µm. The masses of the 6 particle size fractions were determined using a top loading balance (MS3002S; Mettler Toledo, Columbus, OH, USA).

Comparison of division methods for ground oats

Whole oats were ground and divided into three replicate test portions of approximately 100 g using four different dividing methods. Two different grinding scenarios that covered desirable and undesirable particle size fraction distributions were used to produce ground oats. To produce the desirable particle size fraction distribution (i.e. containing more of the smaller particles), 4×1 kg of whole oats were ground using the Retsch mill fitted with a 500 µm sieve. For the undesirable particle size fraction distribution, 4×1 kg of whole oats were ground using the commercial coffee grinder on the coarsest setting. Then 1 kg portions of ground oats from the desirable and undesirable grinding scenarios were each divided using four different methods.

In the first method, 1 kg of ground oats was divided using a riffle divider with 14 alternating channels that moved sample into two directions (H-3966, Humboldt Testing Equipment, Norridge, IL, USA). The riffle divider split samples into two portions. With this equipment, 1 kg of ground oats was placed in a pan whose length was equal to the width of the riffle divider hopper. The pan was tipped over in one motion and two fractions were collected in separate pans whose whole length was equal to the width of the riffle divider hopper. After the first halving of the 1 kg of ground oats, both fractions were recombined and split again. Then one half was selected and divided in multiple iterations using the riffle divider to prepare test portions of approximately 100 g.

In the second method, manual scooping using a metal scoop was used to prepare approximately 100 g test portions. The 1 kg was sampled by taking 10 scoops. Initially 5 scoops

were taken from different locations in the sample container. Remaining sample was mixed by shaking for approximately 30 s and then the remaining 5 scoops were taken from different locations of the container.

For the third division method, a Boerner divider (Seedburo Equipment Company, Des Plaines, IL, USA) was used. Ground oats (1 kg) were placed in the hopper with the spout valve closed. After all samples were added, the valve was opened and two separate pans were used to collect the two fractions produced. After the first halving of the 1 kg of ground oats, both fractions were recombined and split again. Then one half was selected and divided in multiple iterations on the Boerner divider to prepare test portions of approximately 100 g.

For the last method, the rotary sample divider and process described in 'Analysis of particle size distribution of ground oats' was used. The 1 kg sample of ground oats was passed through the rotary sample divider twice. On the second pass, the 10 individual 100 g portions were collected.

The particle size distribution of the ground oats produced by the various dividing methods was determined using mechanical sifting as described above. Three approximately 100 g test portions from each of the four dividing methods were analysed. The division methods for the ground oats were evaluated by considering the mean and standard deviation (n=3) of the percentage of the total sample mass for each particle size fraction obtained from box sifting.

Distribution of mycotoxins and ergosterol amongst particle size fractions

Two samples of oats (2 kg each), portions of which were previously analysed and found to contain a number of mycotoxins, were ground using the Retsch mill fitted with the 500 µm sieve. The ground samples were sifted through a laboratory-scale plansifter described above. The ground samples (one 100 g portion from each sample) were placed on the top sieve and sifted for 90 s. The top sieve was tapped 3 times on each side with a rubber hammer. After tapping, the material remaining on the top sieve (1,180 µm) was collected. Material remaining on top of each of the remaining sieves was collected after successive 90 s periods of sifting and tapping. Material smaller than 110 µm was collected by brushing down the last sieve (110 µm) into the bottom pan. The collected particle size fractions were analysed for mycotoxins and ergosterol. Ultra-high performance liquid chromatography with tandem mass spectrometry (LC-MS/MS) was used to analyse for DON, deoxynivalenol-3-glucoside (DON-3G), 15-acetyl deoxynivalenol (15-ADON), 3-acetyl deoxynivalenol (3-ADON), culmorin (CUL), zearalenone (ZEN), HT-2 toxin, T-2 toxin, nivalenol (NIV), beauvericin (BEA), enniatin A (ENN A), enniatin A1 (ENN A1), enniatin B

(ENN B), enniatin B1 (ENN B1), fumonisin B₁, fumonisin B₂, alternariol (AOH), alternariol methyl ether (AME), altenuene (ALT), tentoxin (TTX), ochratoxin A (OTA), citrinin, aflatoxin B₁, aflatoxin B₂, aflatoxin G₁, and aflatoxin G₂. The method was performed as described in Tittlemier *et al.* (2019).

Briefly, samples were extracted with acetonitrile/water/ acetic acid and diluted in mobile phase. Stable isotope-labelled ¹³C internal standards were added to the diluted sample extract prior to instrumental analysis. Mass spectrometric analysis was performed using electrospray ionisation with polarity switching. Two transitions were monitored and used to identify and quantify each of the mycotoxin analytes. Mycotoxins were quantified using a calibration curve constructed from 7 external standards. Peak areas from quantitation transitions were normalised to the peak area of ¹³C-labelled internal standard during data analysis in order to mitigate matrix effects on quantitation prior to interpolation of analyte concentration from the calibration curve.

Analytes were considered to be identified and were quantified when their retention time was within 0.1 min of the mean retention time in external standards, the ratio of qualifier to quantitation transition was within $\pm 30\%$ of the mean ratio in external standards, and the peak area signal-to-noise ratio was at least 10:1. Gas chromatography with mass spectrometry (GC-MS) was used to analyse samples for ergosterol as described in Tittlemier *et al.* (2019) and based on Chen *et al.* (2016). Ground oats were extracted with 10% KOH in methanol, and further extracted with hexane. The hexane layer was taken to dryness and ergosterol was derivatised with a solution of N-trimethylsilylimidazole: trimethylchlorosilane. The trimethylsilyl derivative of ergosterol was quantitated using matrix-matched calibration standards after analysis by GC-MS in electron impact mode.

Effect of dehulling, steaming, and kilning oats on mycotoxin and ergosterol concentrations

Whole oats (14 samples, 1,500 g each) were dehulled using a laboratory-scale huller (LH-5095, Codema, Maple Grove, MN, USA). Whole oats were processed in the huller for 55 s in batches until the entire 1500 g were completed. After dehulling, the groats were passed through a dockage tester (Carter Day International, Minneapolis, MN, USA) to remove any remaining hulls. Groats and remainders (oat kernels that were not dehulled) were passed through the laboratory-scale huller and dockage tester for a second time. Remainders were manually removed from the groats. Groats, remainders, and hulls were collected, weighed, and stored separately for each oat sample.

A rotary sample divider was used to split groats into two equally sized portions. One portion was used for mycotoxin analysis, and the other was subjected to heat treatment. Groats were steamed and kilned using a vegetable steamer (model VS-30; Salton, Dollard-des-Ormeaux, QC, Canada) and forced air convection lab oven (Isotemp model 838F, Fisher Scientific, Waltham, MA, USA), respectively. Groats were steamed at 100 °C to increase the groat moisture content to 18%. Steamed groats were cooled for 30 min and then placed onto a perforated metal screen tray and exposed to hot air in the forced air oven for a 90 min killing process (45 min at 102 °C, cooling from 102 to 83 °C over 10 min, 10 min at 83 °C, cooling from 83 to 60 °C over 25 min). The moisture content of kilned oats was approximately 9%.

Fractions produced from dehulling (groats, hulls, remainders) and the heat-treated groats were ground using the Retsch mill fitted with the 500 µm sieve and analysed for mycotoxins and ergosterol using the LC-MS/MS and GC-MS methods described above. CUL was included in the analytical method for only 4 samples. The remaining analytes were monitored in the oat fractions from all 14 samples.

Statistical analysis

Statistical analyses were performed using SigmaPlot 13.0 (Systat, San Jose, CA, USA) and a level of significance at $P=0.05$.

3. Results

Comparison of grinders for the comminution of whole oats

The particle size distribution of oats ground using the different grinders and settings listed in Table 1 are shown in Figure 1. Over 50% of ground sample prepared using the Ditting commercial coffee grinder or Romer mill did not pass through the 1,180 µm sieve of the box sifter. As expected, the 'coarse' grind produced less smaller particles than the 'fine' grind on the Ditting grinder. However, for the Romer mill there was no apparent difference in particle size distributions between the coarse and fine grind settings.

The Retsch and Perten mills produced a more desirable particle size distribution; that is, a larger proportion of the particles produced by these mills were smaller. The percentage of ground sample that was small enough to pass through the 1,180 µm sieve of the box sifter ranged from 89% to 100% for these grinders. The majority of ground oats produced by these grinders was in fractions smaller than 475 µm.

There was also noticeable improvement in the particle size distribution as sieves with smaller opening sizes were used in the Retsch and Perten grinders. The biggest improvement

Figure 1. Particle size distribution obtained after grinding whole oats using various grinders and settings. Information in parentheses after each grinder brand indicates the setting or sieve opening size (μm) used.

occurred with the Retsch mill with the 500 μm as compared to the 750 μm sieve. Material large enough that it couldn't pass through the 1,180 μm sieve of the box sifter was eliminated and the material greater than 475 μm in size was reduced from 42 to 33%.

As with the Retsch, there was more ground oats as smaller particle sizes when the sieve on the Perten was changed from 750 to 500 μm . The amount of material that was not small enough to pass through the 475 μm sieve decreased from 26 to 16%.

Grinding of 1 kg of whole oats, plus cleaning the grinder to prepare for a subsequent sample, took over 30 min for the Romer and Perten grinders (Table 1). These grinders both heated up quickly while the oats were being ground. In order to prevent the sample from jamming the grinder, grinding was stopped at frequent intervals to allow the equipment to cool down. Since the capacity of the feeder on the Perten grinder was approximately 500 g, the operator had to add multiple portions to the feeder to grind the entire 1 kg. The whole oats had to also be assisted through the Perten feeder to facilitate movement into the grinder. The whole oats were pushed through the feeder with a metal spatula and the feeder was tapped to prevent blockage. Without this assistance the whole oats would remain in the hopper and would not fall into the grinder. In addition, ground oats were retained on the sieves of the Retsch grinder, but this material was easily accessible and could be removed by tapping the sieve into the ground sample collection container.

Comparison of division methods for ground oats

Results from the investigation into the ability of various methods to accurately and precisely divide ground oats is presented in Figure 2(A). Two sets of grinding conditions were selected from the comparison of grinders in order to cover ideal (Retsch grinder fitted with the 500 μm sieve) and less ideal particle size fraction distributions (Ditting commercial coffee grinder on the coarsest setting). For the sample ground using the Ditting grinder, all four dividing methods produced test portions with consistent size fraction distributions. The mean, as well as the variance of the mean percent mass of each particle size fraction, was not significantly different amongst the six size fractions produced by the Ditting grinder (Welch's ANOVA assuming unequal variances; $P > 0.05$).

However, the four dividing methods did not produce consistent size fraction distributions when the oats sample ground using the Retsch grinder was divided. The variance of the mean percent mass of particle size fractions significantly differed amongst the dividing methods. Significant differences in variances were observed for the 110 to 180 μm , >180 to 300 μm , and >475 to 1,180 μm particle size fractions. The variance from the scooping method was always amongst the highest. The rank order of variance from the other methods was not consistent.

In addition, there were differences amongst the size fraction distributions themselves. As illustrated in Figure 2(B), the differences amongst distributions are small. The differences occur due to lower amounts of the largest particles, and higher amounts of the medium particles, in the ground oats divided using the rotary sample divider. Over 68% of the sample divided using the rotary sample divider was 110 to 475 μm in diameter, as compared to 63–65% of the samples divided using the other methods. Only 25% of the sample divided using the rotary sample divider was >475 μm in diameter, as compared to 28–31% of the samples divided using the other methods.

Distribution of mycotoxins and ergosterol amongst particle size fractions

Out of the 25 mycotoxin analytes in the testing method, only 11 were detected in at least one of the two fractionated oat samples. BEA, ENN B, and TTX were present in both samples. NIV was only in sample 1, and OTA, DON, DON-3G, ZEN, AOH, AME, and ENN A were only in sample 2. Ergosterol was present in both samples. The relative amounts of mycotoxins present in the two fractionated oat samples are displayed in Figure 3. For the detected mycotoxins, concentrations were set to half the limit of quantitation when one of the particle size fractions from a sample did not contain quantifiable concentrations. This occurred with NIV in the <110 μm fraction and ENN B

Figure 2. Uniformity of ground oats particle size distribution after division by different methods. Bars and whiskers indicate mean percentage $\pm$ standard deviation of total sample mass ($n=3$) of ground oats particle size fractions determined by box sifting. (A) Oats ground using the Ditting commercial coffee grinder on the 'coarse' setting. (B) Oats ground using the Retsch mill fitted with a 500 μm sieve.

and DON-3G in the $>475\ \mu\text{m}$ fraction. In addition, ZEN and ENN A were not included in Figure 3 because they were only detected in the >110 to $180\ \mu\text{m}$ and >180 to $300\ \mu\text{m}$ fractions.

Effect of dehulling oats on mycotoxin and ergosterol concentrations in oat fractions

Table 2 summarises the concentrations of ergosterol and mycotoxin measured in the oat fractions produced by the dehulling process. Median concentrations were nominally higher in hulls as compared to groats for ergosterol and most

Figure 3. Distribution of mycotoxins and ergosterol amongst particle size fractions of ground oats. AME = alternariol methyl ether; AOH = alternariol; BEA = beauvericin; DON = deoxynivalenol; DON-3G = DON-3-glucoside; ENN B = enniatin B; NIV = nivalenol; OTA = ochratoxin A; TTX = tentoxin.

mycotoxins. The exceptions were BEA, ENN A1, AOH, and AME, where concentrations in the groats appeared to be higher than in the hulls.

ZEN, NIV, HT-2, and 3-ADON occurred infrequently and at low concentrations in the samples. These mycotoxins were only quantifiable in hulls, and were below the limit of quantification in groats for all samples.

Processing factors were estimated as the ratio of mycotoxin concentrations in groats or hulls to the concentration in whole oats, back-calculated from concentrations in oat fractions and the masses of the oat fractions. Calculations in whole oats were estimated in this manner as opposed to analysing a separate portion of whole oats in order to avoid variation due to the heterogeneity of mycotoxins that has been noted in whole oats (Whitaker *et al.*, 2015a) and other cereal grains (Tittlemier *et al.*, 2012).

For DON and OTA, the median ratios of groats to whole oats concentrations were 0.13 and 0.55, respectively. These ratios reflected the decreases in concentration in groats as compared to whole oats. The median ratios of hulls to whole oats concentrations were higher, at 3.5 for DON and 3.2 for OTA.

Figure 4 illustrates the relative distribution of mycotoxin and ergosterol amongst dehulled oat fractions for selected analytes. While groats and hulls accounted for a median of 71 and 28% of the mass of dehulled whole oats, the majority of ergosterol and mycotoxin mass was associated with hulls. For the mycotoxins, medians of 48% (DON-3G) to 100% (HT-2

Table 2. Concentrations of mycotoxins and ergosterol in oat processing fractions.

Analyte ¹	Fraction	Samples >LOQ ² (%)	Median (µg/kg)	Range (µg/kg)	Analyte ¹	Fraction	Samples >LOQ ² (%)	Median (µg/kg)	Range (µg/kg)
ergosterol	groats	100	640	61-1,870	ENN B1	groats	43	24	<16-24
	hulls		3,640	546-19,800		hulls		27	<16-79
	remainders ³		5,550	5,380-9,330		remainders		50	<16-70
CUL	groats	100	31	12-50	ZEN	groats	43	<20	- ^a
	hulls		224	135-397		hulls		55	<20-85
	remainders		162	86-420		remainders		37	<20-43
DON	groats	93	214	<30-600	OTA	groats	29	3.6	<0.6-5.2
	hulls		856	<30-7,859		hulls		7.3	<0.6-16
	remainders		2,699	<30-7,847		remainders		<0.6	-
TTX	groats	93	19	<5-139	NIV	groats	29	<60	-
	hulls		49	<5-160		hulls		77	<60-167
	remainders		25	<5-122		remainders		76	<60-76
BEA	groats	93	60	<2-83	ENN A	groats	21	7.8	<2-7.8
	hulls		28	2.6-71		hulls		8.7	<2-14
	remainders		9.1	<2-104		remainders		2.2	<2-5.3
ENN B	groats	71	33	<16-40	HT-2	groats	21	<60	-
	hulls		107	<16-259		hulls		70	<60-137
	remainders		43	<16-289		remainders		<60	-
DON-3G	groats	71	179	<50-252	3-ADON	groats	14	<50	-
	hulls		244	<50-555		hulls		182	<50-264
	remainders		259	<50-841		remainders		<50	-
AOH	groats	71	28	<4-50	¹ AME = alternariol methyl ether; AOH = alternariol; BEA = beauvericin; CUL = culmorin; DON = deoxynivalenol; 3-ADON = 3-acetyldeoxynivalenol; DON-3G = DON-3-glucoside; ENN A = enniatin A; ENN A1 = enniatin A1; ENN B = enniatin B; ENN B1 = enniatin B1; HT-2 = HT-2 toxin; NIV = nivalenol; OTA = ochratoxin A; TTX = tentoxin; ZEN = zearalenone. ² LOQ = limit of quantification. ³ Remaining oat kernels not dehulled by laboratory-scale huller.				
	hulls		25	<4-305					
	remainders		37	<4-53					
AME	groats	57	32	<4-32					
	hulls		14	<4-124					
	remainders		19	<4-32					
ENN A1	groats	50	11	<5-11					
	hulls		8.2	<5-17					
	remainders		11	<5-12					

toxin and 3-ADON) of mass was associated with hulls; 67% of the mass of ergosterol was associated with hulls.

Effect of heat treatment on mycotoxin and ergosterol concentrations in groats

Changes in concentration of ergosterol and selected mycotoxins after steaming and kilning of groats are shown in Figure 5. Ergosterol concentrations were consistently higher in steamed and kilned groats as compared to the untreated groats. Absolute concentration increases ranged from 0.86 to 1.21 mg/kg in the 14 samples analysed. On the other hand, DON and DON-3G concentrations were consistently lower in steamed and kilned as opposed to untreated groats. Decreases in concentration were observed in 6 of the 7 occurrences of DON (mean decrease of 27%) and 4 out of the 6 occurrences of DON-3G (mean decrease of 20%) in the samples after steaming and kilning. There were no consistent changes in TTX concentrations after

steaming and kilning. There were too few occurrences of quantifiable concentrations of the other mycotoxins to determine if changes were due to heat treatment or sample heterogeneity.

4. Discussion

Comparison of grinders for the comminution of whole oats

The rotor beater type grinders (Retsch and Perten) produced more of the smaller particles than the burr mill type grinders (coffee grinders and Romer mill), and therefore produced more desirable particle size distributions. In general, more smaller particles are preferred after comminution as this will decrease the heterogeneity of mycotoxins in a sample and the variation due to sampling (Smith, 2001). The burr type mills evaluated have limited options for adjusting particle sizes produced, as adjustments are done by modifying the space between the rotating abrasive surfaces. The rotor

Figure 4. Median percentage of mycotoxin and ergosterol mass distributed amongst dehulled oat fractions. (A) Oat mass; (B) ergosterol; (C) deoxynivalenol-3-glucoside; (D) deoxynivalenol; (E) alternariol; (F) tentoxin; (G) beauvericin; (H) enniatin B.

beater type grinders use sieves of various opening sizes to adjust the particle size distribution of ground grain.

Oat hulls were more difficult to comminute than endosperm of the kernels. Visually, it could also be seen that for the burr mill type grinders, the larger particle sizes tended to be oat hulls. The shearing action of this type of grinder produced large slivers of hulls amongst the smaller endosperm particles.

We chose to use the Retsch fitted with the 500 µm sieve for processing of oat samples for subsequent mycotoxin and ergosterol analyses for two main reasons. First, this equipment produced a desirable particle size distribution. Approximately 67% of ground oats comminuted in this system were small enough to pass through the 475 µm sieve on the box sifter. Fragments of hulls were still visible in the ground oats, but they were smaller than the hull fragments produced by the other grinders. Secondly, the time to grind 1 kg of whole oats and clean the grinder in preparation for the next sample was only 6 min. The chosen grinder system provided a good balance between maximising the amount of desirable particle sizes and minimising the time needed to comminute sample and clean the grinder.

Comparison of division methods for ground oats

All of the rotary and gravity fed stationary dividers assessed in this study outperformed the manual scooping method for dividing ground oats. Manual scooping was the method the least able to maintain a consistent particle size distribution amongst replicate test portions taken from the same finely ground material. When subsampling the coarsely ground oats (in which over 90% of the material had a diameter larger than 475 µm), all division methods performed the same.

There was indication of bias in the particle size distributions amongst the division methods for finely ground oats. No bias was apparent during the division of the coarsely ground oats. However, the least biased division method could not be determined without knowing the ‘true’ distribution. Even though the division method appeared to affect the particle size distribution, as well as the variance of the distribution for replicate test portions, the choice of division may not have practical effects on the analysis of mycotoxins in ground oats. Using the results from this work, perturbations in the particle size distribution due to bias and/or variance from the sample division method would cause only a small change in mycotoxin concentration in a sample of ground oats.

The particle size distributions from the manual scooping and rotary sample divider methods (Figure 2B) were considered along with the distribution of OTA and DON in the particle size fractions as shown in Figure 3. Assuming the concentrations of OTA and DON are constant within a particle size fraction, the estimated concentrations of OTA (1.79 vs 1.77 µg/kg) and DON (752 vs 769 µg/kg) in the manual scooping and rotary sample divided test portions only vary up to 2%. This small difference would be obscured by the variability due to the analytical test method, which has been reported at a relative standard deviation of 7-9% for OTA (Whitaker *et al.*, 2015a,b) and 22% for DON at similar concentrations (Whitaker *et al.*, 2000).

The impact of a method for the division of ground oats is minimal in this work, but analysts should separately

Figure 5. Change in ergosterol and mycotoxin concentration after steaming and kilning groats. The percent change was calculated as the difference in concentrations between untreated and heat-treated groats, divided by the concentration in untreated groats. (A) ergosterol; (B) tentoxin; (C) deoxynivalenol; (D) deoxynivalenol-3-glucoside; (E) culmorin. Instances of no bars in (B-D) indicate that analyte was not detected in untreated groats. Only samples 1 through 4 were analysed for ergosterol and culmorin.

estimate or assess the impact for their own work situations. The greater the differences in mycotoxin concentration amongst the particle size fractions, the greater will be the impact of variations in particle size distributions on measured mycotoxin concentrations.

Distribution of mycotoxins and ergosterol amongst particle size fractions

The aim of this experiment was not to determine where the mycotoxins were located within oat kernels, but to investigate the relationship to particle size in order to gauge if and how grinding and dividing processes may affect

mycotoxin analysis if particle size distribution changes during sub-sampling. If mycotoxins and ergosterol were evenly distributed amongst the particle size fractions, the analyte distributions would mirror the sample mass distribution bar in Figure 3. Deviations of analyte distribution from the sample mass distribution indicate otherwise. These differences in analyte distribution amongst particle size fractions could reflect that there is a gradient of analytes within kernels. The differences could also reflect differences in extractability from different particle sizes. This has been observed before (Garcia-Fonseca *et al.*, 2010; Schatzki and Toyofuku, 2003; Tittlemier *et al.*, 2012).

The only compound where a majority (>50%) of total mycotoxin mass was in one particle size fraction was OTA. Other analytes were distributed more amongst all of the particle size fractions. A similar presence of the majority of OTA in the smallest particle size fraction has been observed for wheat (Tittlemier *et al.*, 2012).

The results from this experiment suggest that the analysis of OTA may be more sensitive towards the methods of sample preparation that could affect the presence of particles <110 µm in diameter than other mycotoxins. Comminution, as well as processes involved with handling and/or sub-sampling, can all affect the proportion of smallest particles present and therefore should be assessed to avoid biasing particle size fraction distributions of samples and sub-samples.

Effect of dehulling oats on mycotoxin and ergosterol concentrations in oat fractions

This study contains the first reports of the fate of OTA, CUL, ZEN, AOH, AME, TTX, ENNs and BEA in oats, and showed that the majority of ergosterol and mycotoxins is associated with hulls. Quantifying the magnitude of the effect of processing was limited, because of the low frequency of occurrence and low concentrations of these mycotoxins in the naturally infected samples used in this study. However, the predominance of mycotoxins in oat hulls with a much lower presence, or even absence, in groats was consistent for the quantifiable analytes.

The large decrease in DON, HT-2, and NIV concentrations in groats due to dehulling observed in this study was consistent with past research. For example, reductions on the order of 88 to 94% were reported for DON (Ivanova *et al.*, 2016; Scudamore *et al.*, 2007). The median reduction in the current study was 87%. HT-2 and NIV were also reduced to a similar extent in other studies (Adler *et al.*, 2003; Ivanova *et al.*, 2016; Scudamore *et al.*, 2007). In the current study, HT-2 and NIV were only detected in the hull fraction. None of the groats analysed contained quantifiable amounts. Concentrations of ergosterol in groats were approximately half those in whole oats, which was also consistent with previous research. The median ratio of 0.47 for ergosterol concentration in groats to whole oats was very similar to the ratio of 0.50 observed by Adler *et al.* (2003).

These results suggest fungi have limited potential to penetrate the hulls and colonise the endosperm or the germ. Ivanova *et al.* (2016) reported that fungal DNA was concentrated in oat fractions representing hulls and bran and outer layers of endosperm, which is in line with the mycotoxin distributions observed in the current study. The current results also imply that most mycotoxins are not readily translocated from hulls to oat kernel.

Notably though, DON-3G and TTX tended towards higher occurrence in groats than the other analytes. A shift in occurrence of DON-3G towards groats was noted by Ivanova *et al.* (2016) as well, and raises questions regarding if there are specific areas on or within an oat kernel of higher activity for transforming DON to DON-3G. TTX has not been previously reported in oat fractions.

While dehulling in the production of oat-containing foods would have a beneficial effect of minimising consumers' exposure, there are potentially problematic implications for the use of oat hulls in feed. Some of the whole oats used in the dehulling experiment generated hulls containing DON at concentrations higher than the proposed Canadian guideline of 5 mg/kg for DON in cereals and cereal by-product single ingredient feeds for calves, lactating dairy animals, and swine (Canadian Food Inspection Agency, 2017). It would be prudent to monitor oat hulls to ensure compliance with feed guidelines and to avoid harmful effects on livestock.

The association of mycotoxins with hulls could also impact exposure assessments and regulations. While oat hulls are used in animal feed, they are not used in food. One of the first steps in processing oats for use in food is dehulling, therefore accurate measures of consumers' exposure to mycotoxins via oat consumption should be based on concentrations in groats and not whole oats. Basing exposure assessments on the consumption of whole oats will overestimate exposure. The degree of overestimation would vary according to the mycotoxin considered. Based on the results of this study, exposure to DON and OTA could be overestimated by factors of 8× and 2×, respectively if whole oat concentrations were considered as opposed to concentrations in groats.

Effect of heat treatment on mycotoxin and ergosterol concentrations in groats

Heat treatment of groats appeared to increase the fungal biomass, as ergosterol concentrations were consistently higher in oats that were steamed and kilned as compared to untreated groats. However, no increases in mycotoxin concentration were observed, suggesting the increase in fungal biomass was for non-toxicogenic species, or that conditions were not conducive to mycotoxin production.

Changes in TTX concentrations for the 11 samples that contained quantifiable concentrations were not consistent; 5 samples showed increased concentrations in the heat-treated groats, whereas 6 samples showed decreased concentrations. Combined with the low concentrations, this suggests the variation in concentrations observed were due to sample heterogeneity with respect to TTX.

In addition, decreases in DON and DON-3G concentrations were observed. The consistency of the lower concentrations in the heat-treated groats suggests that the apparent differences were not simply sampling artefacts, as hypothesised for TTX. Reductions in DON content have been reported for wheat treated with super-heated steam for short periods of time (Cenkowski *et al.*, 2007). Reductions of up to 52%, observed at a temperature of 185 °C for 2–6 s, were attributed to thermal degradation. Smaller reductions in DON content observed at lower temperatures of 135 °C and 110 °C treated for 3–10 s were hypothesised to be due to a ‘washing effect’. In the current study, steaming occurred at 100 °C following by kilning at 102 °C for 45 min, suggesting that the prolonged exposure to heat and steam reduced DON and DON-3G content of groats.

5. Conclusions

Some of the steps involved in processing whole oats for analysis, and for processing for food and feed use did have an effect on mycotoxin concentrations. With respect to processing for analysis, the type of grinder and settings used to comminute whole oats affected the particle size distribution of ground sample. Rotor beater type grinders produced the more desirable finer ground samples as compared to burr type mills. When sub-dividing ground sample, the type of division method used is not expected to have a practical effect on mycotoxin analysis. With respect to processing oats for use, dehulling removed the majority of ergosterol and mycotoxins whereas heat treatment of groats increased fungal load but decreased the presence of some mycotoxins. These effects should be taken into account by analysts preparing whole oats for mycotoxin analysis, as well as regulators assessing dietary exposure and potential risk from the consumption of food and feed.

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Conflict of interest

The authors declare no conflict of interest.

References

Adler, A., Lew, H., Moudry, J., Sterba, Z., Vratilova, K., Edinger, W., Brodacz, W. and Kiendler, E., 2003. Microbiological and mycotoxicological quality parameters of naked and covered oats with regard to the production of bran and flakes. *Die Bodenkultur* 54: 41–49.

Bouphasiri, C. and Verchomin, S., 2005. A case study of the Canadian oat market: the evolution from the central desk system to the open market. Alberta Agriculture, Food and Rural Development, Edmonton, Canada.

Campbell, H., Choo, T.M., Vigier, B. and Underhill, L., 2002. Comparison of mycotoxin profiles among cereal samples from eastern Canada. *Canadian Journal of Botany* 80: 526–532. <https://doi.org/10.1139/b02-055>

Cenkowski, S., Pronyk, C., Zmidzinska, D. and Muir, W.E., 2007. Decontamination of food products with superheated steam. *Journal of Food Engineering* 83: 68–75. <https://doi.org/10.1016/j.jfoodeng.2006.12.002>

Canadian Food Inspection Agency, 2017. Proposal – contaminant standards for aflatoxins, deoxynivalenol, fumonisins, ergot alkaloids and *Salmonella* in livestock feeds. Available at: <https://tinyurl.com/rpsby8z>

Chen, W., Turkington, T.K., Lévesque, C.A., Bamforth, J.M., Patrick, S.K., Lewis, C.T., Chapados, J.T., Gaba, D., Tittlemier, S.A., MacLeod, A. and Gräfenhan, T., 2016. Geography and agronomical practices drive diversification of the epiphytic mycoflora associated with barley and its malt end product in western Canada. *Agriculture, Ecosystems and Environment* 226: 43–55. <https://doi.org/10.1016/j.agee.2016.03.030>

Clear, R.M., Patrick, S.K. and Gaba, D., 2000. Prevalence of fungi and fusariotoxins on oat seed from western Canada, 1995–1997. *Canadian Journal of Plant Pathology* 22: 310–314. <https://doi.org/10.1080/07060660009500480>

Garcia-Fonseca, S., Ballesteros-Gomez, A., Rubio, S. and Perez-Bendito, D., 2010. Supramolecular solvent-based microextraction of ochratoxin A in raw wheat prior to liquid chromatography-fluorescence determination. *Journal of Chromatography A* 1217: 2376–2382. <https://doi.org/10.1016/j.talanta.2015.11.014>

Grusie, T., Cowan, V., Singh, J., McKinnon, J. and Blakley, B., 2017. Correlation and variability between weighing, counting and analytical methods to determine ergot (*Claviceps purpurea*) contamination of grain. *World Mycotoxin Journal* 10: 209–218. <https://doi.org/10.3920/WMJ2016.2174>

Ivanova, L., Sahlström, S., Rud, I., Uhlig, S., Fæste, C.K., Eriksen, G.S. and Divon, H.H., 2016. Effect of primary processing on the distribution of free and modified *Fusarium* mycotoxins in naturally contaminated oats. *World Mycotoxin Journal* 10: 73–88. <https://doi.org/10.3920/WMJ2016.2092>

Kuchenbuch, H.S., Becker, S., Schulz, M., Cramer, B. and Humpf, H.-U., 2018. Thermal stability of T-2 and HT-2 toxins during biscuit- and crunchy muesli-making and roasting. *Food Additives and Contaminants Part A* 35: 2158–2167. <https://doi.org/10.1080/19440049.2018.1530456>

Lee, H.J., Dahal, S., Perez, E.G., Kowalski, R.J., Ganjyal, G.M. and Ryu, D., 2017. Reduction of ochratoxin A in oat flakes by twin-screw extrusion processing. *Journal of Food Protection* 80: 1628–1634. <https://doi.org/10.4315/0362-028X.JFP-16-559>

Martos, P.A., Thompson, W. and Diaz, G.J., 2010. Multiresidue mycotoxin analysis in wheat, barley, oats, rye and maize grain by high performance liquid chromatography-tandem mass spectrometry. *World Mycotoxin Journal* 3: 205–223. <https://doi.org/10.3920/WMJ2010.1212>

- Schatzki, T.F. and Toyofuku, N., 2003. Sample preparation and presampling of pistachios. *Journal of Agricultural and Food Chemistry* 51: 6068-6072. <https://doi.org/10.1021/jf0301955>
- Schmidt, H.S., Becker, S., Cramer, B. and Humpf, H.-U., 2017. Impact of mechanical and thermal energies on the degradation of T-2 and HT-2 toxins during extrusion cooking of oat flour. *Journal of Agricultural and Food Chemistry* 65: 4177-4183. <https://doi.org/10.1021/acs.jafc.7b01484>
- Scudamore, K.A., Baillie, H., Patel, S. and Edwards, S.G., 2007. Occurrence and fate of *Fusarium* mycotoxins during commercial processing of oats in the UK. *Food Additives and Contaminants* 24: 1374-1385. <https://doi.org/10.1080/02652030701509972>
- Seitz, L.M., Mohr, H.E., Burroughs, R. and Sauer, D.B., 1977. Ergosterol as an indicator of fungal invasion in grains. *Cereal Chemistry* 54: 1207-1217.
- Smith, P.L., 2001. A primer for sampling solids, liquids, and gases. American Statistical Association and the Society for Industrial and Applied Mathematics, Philadelphia, PA, USA.
- Tamburic-Ilincic, L., 2010. *Fusarium* species and mycotoxins associated with oat in southwestern Ontario, Canada. *Canadian Journal of Plant Science* 90: 211-216. <https://doi.org/10.4141/cjps09139>
- Tittlemier, S.A., Blagden, R., Chan, J., Gaba, D., McKendry, T., Pleskach, K. and Roscoe, M., 2019. *Fusarium* and *Alternaria* mycotoxins in Canadian wheat and durum harvest samples. *Canadian Journal of Plant Pathology* 41: 403-414. <https://doi.org/10.1080/07060666.1.2019.1592784>
- Tittlemier, S.A., Blagden, R., Chan, J., Roscoe, M. and Pleskach, K., 2020. A multi-year survey of mycotoxins and ergosterol in Canadian oats. *Mycotoxin Research* 36: 103-114. <https://doi.org/10.1007/s12550-019-00373-9>
- Tittlemier, S.A., Roscoe, M., Kobialka, C. and Blagden, R., 2012. Considerations in the preparation of laboratory samples for the analysis of ochratoxin A in wheat. *World Mycotoxin Journal* 5: 107-116. <https://doi.org/10.3920/WMJ2012.1393>
- Tittlemier, S.A., Varga, E., Scott, P.M. and Krska, R., 2011. Sampling of cereals and cereal-based foods for the determination of ochratoxin A: an overview. *Food Additives and Contaminants Part A* 28: 775-785. <https://doi.org/10.1080/19440049.2011.559278>
- Whitaker, T.B., Hagler Jr., W.M., Giesbrecht, F.G. and Johansson, A.S., 2000. Sampling, sample preparation, and analytical variability associated with testing wheat for deoxynivalenol. *Journal of AOAC International* 83: 1285-1292.
- Whitaker, T.B., Slate, A.B., Nowicki, T. and Giesbrecht, F.G., 2015a. Variability and distribution among sample test results when sampling unprocessed oat lots for ochratoxin A. *World Mycotoxin Journal* 8: 511-524. <https://doi.org/10.3920/WMJ2014.1858>
- Whitaker, T.B., Slate, A.B., Nowicki, T.W. and Giesbrecht, F.G., 2015b. Variability and distribution among sample test results when sampling unprocessed wheat lots for ochratoxin A. *World Mycotoxin Journal* 9: 163-178. <https://doi.org/10.3920/WMJ2015.1970>
- Yan, W., Pageau, D., Martin, R., Cumiskey, A. and Blackwell, B., 2017. Is deoxynivalenol contamination a serious problem for oat in eastern Canada? *Crop Science* 57: 88-98. <https://doi.org/10.2135/cropsci2016.04.0263>