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Fate of Regulated, Masked, Emerging Mycotoxins and Secondary Fungal Metabolites during different large-scale maize dry-milling processes

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Abstract

The worldwide consumption of maize for food is increasing, since it is used as an ingredient for several foods and in particular for gluten-free products, whose consumption is rising.

In temperate areas, the main limitation to the use of maize in the food chain is its contamination by mycotoxins. Limited information is available on the fate of masked, modified and emerging mycotoxins or of other secondary fungal metabolites in maize products and by-products. For this reason, 3 maize lots, obtained in different growing seasons, were processed using two different degermination processes, a dry-degermination system or a tempering-degermination one, in order to compare the interaction between mycotoxins and the dry-milling management system. Whole grain before and after cleaning, and all the products and the by-products were sampled twice for each lot and were subjected to a multi-mycotoxin LC-MS/MS analysis. More than 30 mycotoxins and other fungal metabolites, including masked or modified forms, co-occurred in all the maize milling fractions. Grain cleaning reduced all the detected fungal metabolites by 1.2-2 times, compared to the grain before cleaning. Animal feed flour showed the highest content of almost all the mycotoxins and fungal metabolites, with a consequent negative impact on animal health. Overall, the sum of the 3 food-grade endosperm fractions from tempering-degermination (flaking grits, medium and small hominy grits) resulted in a lower contamination than those obtained from the dry-degermination (pearl meal, break meal and maize flour). Moreover, considering that for all the mycotoxins and fungal metabolites an inverse relationship with particle size was observed, flaking grits represented the healthiest maize products with the least contamination level, while the abatement was always lower for maize flour.

Furthermore, the metabolites were variably redistributed in the maize fractions. The total aflatoxins, kojic acid, deoxynivalenol and its modified form, culmorin, and its associated forms, butenolide, fusaproliferin, fusaric acid, fusarinolic acid and, in some cases, zearalenone and its modified forms, and fusarin C were found to be concentrated significantly in the germ. Moreover, the total

aflatoxins, deoxynivalenol-3-glucoside, fusarinolic acid, fusarin C, moniliformin and butenolide had a greater permanence in the maize food fractions and a weaker decontamination, both of which point to a higher risk of exposure for the end consumers.

The co-occurrence of a such a high number of mycotoxins and fungal metabolites and their different fates during the dry-milling process have never been described before and could be useful for future risk assessment studies to correctly assess the risk of exposure to such substances. Moreover, the continuous exposure to these mycotoxins and fungal metabolites should be considered in particular for consumers in the many parts of the world where maize is a staple food, and where it is used for the baby food supply chain and for the celiac population in developed countries, due to the high consumption of maize gluten-free products.

KEYWORDS: aurofusarin; beauvericin; culmorin; deoxynivalenol-3-glucoside; fusaric acid; fusarin C; moniliformin; zearalenone-sulphate.

ABBREVIATIONS

3-ADON, 3-acetyldeoxynivalenol; 15-ADON, 15-acetyldeoxynivalenol; 5-OH-CULM, 5-hydroxy-culmorin; 15-OH-CULM, 15-hydroxy-culmorin; 15-OH-culmoron, 15-hydroxy-culmoron; α-ZEA-ol, alpha-zearalenol; β-ZEA-ol, beta-zearalenol; AFs, Aflatoxins; AF_{TOT}, Total aflatoxins, sum of AFB_1, AFB_2, AFG_1 and AFG_2; ALS, altersetin; AME, alternariol methyl ether; ANOVA, Analysis of variance; AOH, alternariol; AUR, aurofusarin; BEA, beauvericin; BIK, bikaverin; BUT, butenolide; CAC, Codex Alimentarius Commission; CULM, culmorin; $CULM_{TOT}$, Total culmorin forms, sum of CULM, 5-OH-CULM, 15-OH-CULM, 15-OH-culmuron; DAS, diacetoxyscirpenol; DD, Dry-Degermination; DON, Deoxynivalenol; DON-3-G, deoxynivalenol-3-glucoside; DON_{TOT}, Total deoxynivalenol forms, sum of DON, DON-3-G, 3-ADON and 15-ADON; EC, European Commission; EFSA, European Food Safety Authority; ENNs, enniatins; ENN_{TOT}, Total enniatins, sum of ENN A, A_1, B and B_1; EQU, equisetin; ESI, Electrospray Ionization; FA_{TOT}, Total fumonisins A, sum of FA_1, FA_2; FBs, Fumonisins B; FB_{TOT}, Total fumonisins B, sum of FB_1, FB_2, FB_3 and FB_4; FnA, fusarinolic acid; FSA, fusaric acid; FUS, fusaproliferin; HFB_1, hydorlized fumonin B_1; JECFA, Joint FAO/WHO Expert Committee on Food Additives; LD_{50}, Lethal Dose 50%; LOD, limit of detection; LOQ, limit of quantification; MON, moniliformin; NIV, nivalenol; OTA, ochratoxin A; REGWF, Ryan-Einot-Gabriel-Welsh F post-hoc test; SD, Standard Deviation; TD, Tempering-Degermination; TeA, tenuazonic acid; TEN, tentoxin; ZEA, Zearalenone; ZEA-S, zearalenone-sulphate; ZEA_{TOT}, total zearalenone forms sum of ZEA, ZEA-S, α-ZEA-ol and β-ZEA-ol.

1. Introduction

Maize is the main cereal grain produced worldwide, although it ranks third as a staple food, after wheat and rice. The consumption of this crop has recently increased in developed countries, as it is used as an ingredient for breakfast cereals, snacks, dietetic products, and in particular for baby food and gluten-free food formulations, whose consumption is rising (Rai et al., 2018).

Unfortunately, maize can be colonised competitively by several spoilage fungi of the *Fusarium*, *Aspergillus*, *Alternaria* and *Penicillium* species, which are capable of producing a large variety of mycotoxins and other secondary fungal metabolites as a result of fungal ear rot on maize ears (Marin et al., 2012), which in turn lead to a negative impact on the safety and quality of this agricultural commodity. In this regard, a recent worldwide study on the contamination of food-crops with mycotoxins has pointed out that 60-80% of food crops are contaminated with mycotoxins (Eskola et al., 2019).

Approximately 400 mycotoxins or potential risky fungal metabolites are known to date throughout the world (Berthiller et al., 2007), but aflatoxins (AFs), fumonisins B (FBs), deoxynivalenol (DON), zearalenone (ZEA) and ochratoxin A (OTA) are the only mycotoxins that are generally regulated and monitored (Binder, 2007). The other mycotoxins, which are less known from a scientific point of view and which may co-occur with the regulated mycotoxins, have become part of the so-called “masked”, “modified” and “emerging” mycotoxins or other secondary fungal metabolites (Streit et al., 2013). Masked mycotoxins are plant metabolites of mycotoxins, or according to Rychlik et al.’s (2014) systematic definition “biologically modified” mycotoxins, whose chemical modifications, introduced by the plant’s metabolism, have the potential to affect both their toxicity and analytical detectability. Among the group of masked mycotoxins, deoxynivalenol-3-glucoside (DON-3-G) and zearalenone-sulphate (ZEA-S) and are the most commonly found in food and feeds. Their toxicological properties are currently being investigated, and mainly involve the conversion of DON-3-G to DON and ZEA-S to ZEA by microbiota of the

intestinal tract (Dall'Erta et al., 2013). Emerging mycotoxins are a group of chemically diverse mycotoxins, for which, to date, no regulations exist, and ongoing risk assessment studies are still in progress. Aflatoxin precursors, ergot alkaloids, enniatins (ENNs), beauvericin (BEA) and moniliformin (MON) are those that are more commonly mentioned in this group (Jestoi, 2008). Moreover, there is no clear indication of the toxicity of the other secondary fungal metabolites that are frequently found in cereals, such as aurofusarin (AUR) and culmorin (CULM), and they are still the subject of detailed studies.

Since little is known about the toxicological effects of these compounds and limited information is available about the synergistic or additive toxic effects related to their co-presence with the regulated mycotoxins, a higher risk of exposure for the end consumers and health issues could emerge.

Dry-milling is the main industrial process adopted in the maize food chain to obtain hominy grits, maize flours and meals for human consumption. This technology consists of a mechanical kernel processing that creates whole or fractionated products, separated according to their anatomical features, such as bran, germ and endosperm (Gwirtz & Garcia-Casal, 2014). Because of the important role of dry milling in re-distributing contaminants in the different milling products and by-products, several scientific contributions have focused on the fate of the main regulated mycotoxins, such as fumonisins, aflatoxins, deoxynivalenol and zearalenone (Scudamore & Patel, 2000; Brera et al., 2004, 2006; Bullerman & Bianchini, 2007; Castells et al., 2008; Schollenberger et al., 2008; Pietri et al., 2009; Vanara et al., 2009; Burger et al., 2013; Aprodu & Banu, 2015; Bordini et al., 2017; Vanara et al., 2018). Furthermore, there is a lack of information on the fate of masked, modified and emerging mycotoxins and on other secondary fungal metabolites in maize products and by-products (Schollenberger et al., 2008; Scarpino et al., 2020).

To the best of the authors' knowledge, the simultaneous fate and re-distribution of such a high number of mycotoxins, including the regulated, masked, modified, emerging mycotoxins and other secondary fungal metabolites, in maize destined for human consumption, through the application of

the dry-milling process, has not yet been considered in the scientific literature. Moreover, the European Food Safety Authority (EFSA) is continuously engaged in collecting the occurrence data of masked, modified and emerging mycotoxins in food and feeds, in order to establish scientific opinions on their risks for human and animal health. Information on the fate of these contaminants, throughout the supply chain, is an essential information to carry out future risk assessments based on the real exposure of humans and animals, from the raw materials to the final food and feed products.

For this purpose, 3 maize lots, obtained in different growing seasons, were processed using two different degermination processes, a dry-degermination (DD) system and a tempering-degermination (TD) one, in order to compare the interaction between mycotoxins and the adopted dry-milling management process.

2. Material and methods

2.1 Maize milling processes and sampling

The occurrence and distribution of regulated, masked, emerging mycotoxins and secondary fungal metabolites have been investigated by sampling and analysing in 3 different growing seasons (2012, 2013 and 2014), in the same growing area (North West Italy, the province of Turin), a single maize hybrid each year from 3 commercial lots (Pioneer P1547 in 2012 and 2013, Pioneer P0722 in 2014), for food dry milling purposes.

The maize from each lot was milled in two separate dry-milling industrial lines, which were based on different degermination processes. The first line consisted of a dry-milling technology, coupled to a dry-degermination (DD) system, while the dry-milling technology in the second line was based on a tempering-degermination (TD) process. The two processes have been described in detail by Blandino et al. (2017a).

155 Germs and animal feed flour were the main by-products of both processes, and they have expected
156 yields of 10% and 35%, respectively. The maize products of the 3 lots recorded mean yields of 5%,
157 20% and 30% for maize flour, break meal and pearl meal during the DD process and of 7%, 19%
158 and 29% for small, medium and flaking grits, whose different particle sizes are shown in Figure 1,
159 during the TD process.
160 The sampled products of each process represented a lot of origin of about 200 t and were collected
161 during the milling process according to European Commission Regulation (EC) No 401/2006. An
162 aggregate sample was obtained for each milling fraction by carefully blending 40 incremental
163 samples, of 100 g each, which were collected, by means of a dynamic sampling procedure, from
164 opening slits of the plant for a period of 1 hour at regular intervals. All the maize products and by-
165 products of each lot were sampled twice, before and after cleaning, and were collected from both
166 processes (DD and TD), for a total of 72 samples.
167 The samples were stored at -18°C until the multi-mycotoxin analysis was performed.

168 *2.2 Multi-mycotoxin LC-MS/MS analysis*

169 The samples were prepared according to Sulyok et al. (2006). The chromatographic and mass
170 spectrometric parameters of the investigated analytes were described by Malachova et al. in 2014.
171 Quantification was performed on the basis of an external calibration, and the results were corrected
172 for apparent recoveries, as determined in the maize. Fumonisins A were semi-quantified using the
173 response of FB_2. The accuracy of the method was verified by participating in proficiency testing
174 schemes organised by BIPEA (Gennevilliers, France), with 160 out of the 168 results submitted for
175 maize and maize-based feeds exhibiting a z-score of between -2 and 2.

176 *2.3 Statistical analysis*

177 An analysis of variance (ANOVA) was run for each maize lot to compare the mycotoxin
178 contaminations. The raw kernel and the milling fractions of the two dry milling processes (TD and

179 DD) were considered as the independent variables. The mycotoxin concentrations were transformed
180 using the y'=ln(x+1) equation to normalise the residuals. Multiple comparison tests were carried
181 out, according to the Ryan-Einot-Gabriel-Welsh F (REGWF) post-hoc test, on the mycotoxin
182 contamination means of the different dry-milling fractions.
183 SPSS Version 24.0 of the Windows statistical package, (SPSS Inc., 2017) was used for the
184 statistical analysis.
185

3. *Results and Discussion*

As reported in Table 1, the following main regulated, masked, modified, emerging mycotoxins and other secondary fungal metabolites were simultaneously detected in the pre-cleaned whole grain from the maize from the 3 lots processed in the industrial mill during the 2012-2014 period: fumonisins B (total fumonisins B = FB_{TOT} = the sum of FB_1, FB_2, FB_3 and FB_4); fumonisins A (total fumonisins A = FA_{TOT} = the sum of FA_1, FA_2); hydorlyzed fumonin B_1 (HFB_1); fusaric acid (FSA); fusarinolic acid (FnA); fusarin C; bikaverin (BIK); moniliformin (MON); beauvericin (BEA); fusaproliferin (FUS); enniatins (total enniatins = ENN_{TOT} = the sum of ENN A, A_1, B and B_1); total deoxynivalenol forms (DON_{TOT} = the sum of deoxynivalenol (DON), deoxynivalenol-3-glucoside (DON-3-G), 3-acetyldeoxynivalenol (3-ADON) and 15-acetyldeoxynivalenol (15-ADON)); total zearalenone forms (ZEA_{TOT} = the sum of zearalenone (ZEA), zearalenone-sulphate (ZEA-S), alpha-zearalenol (α-ZEA-ol) and beta-zearalenol (β-ZEA-ol)); total culmorin ($CULM_{TOT}$ = the sum of culmorin (CULM), 5-hydroxy-culmorin (5-OH-CULM), 15-hydroxy-culmorin (15-OH-CULM) and 15-hydroxy-culmoron (15-OH-culmoron)); aurofusarin (AUR); butenolide (BUT); diacetoxyscirpenol (DAS); nivalenol (NIV); equisetin (EQU); T-2 toxin; HT-2 toxin; aflatoxins (total aflatoxins = AF_{TOT} = the sum of AFB_1, AFB_2, AFG_1 and AFG_2); kojic acid; alternariol (AOH); alternariol methyl ether (AME); tentoxin (TEN); tenuazonic acid (TeA); altersetin (ALS). As reported in Table 1, the contamination levels of the different mycotoxins and fungal metabolites may vary significantly from year to year in maize and mainly depend on the environmental conditions of each year, which have an impact on the production of these co-occurring compounds by the main fugal causal agents of diseases on maize (Blandino et al. 2017b).

The fate of FB_{TOT} in the milling fractions of the different dry milling processes and maize lots (2012, 2013 and 2014) is reported in Figure 2. On average, in all of the lots and for all the fractions, FB_1 was about the 68% of the FB_{TOT}, FB_2 was 16%, FB_3 was 9% and FB_4 was 7%. The cleaning step on average reduced the FB_{TOT} content by -47%. Overall, the animal feed flour represented the

211 fraction with the highest FB_{TOT} content for all the lots, with a significant increase, that is, of 3.0 and
212 2.8 times, respectively, in the DD and TD processes, compared to the corresponding pre-cleaned
213 whole grain. The germ significantly differed from the pre-cleaned whole grain for all the lots, with
214 the exception of the germ from the DD process of the 2012 lot, and showed mean reductions of -
215 58% (DD) and -45% (TD), with no significant differences between the processes. Within
216 endosperm products, the maize flour, the break meal and the pearl meal (DD process) showed an
217 FB_{TOT} decrease, compared to the pre-cleaned whole grain, of -24%, -78% and -80%, respectively,
218 while the small, medium hominy grits and flaking grits (TD process) showed decreases of -81% and
219 -89% and -95%, respectively. Decontamination was greater in TD process than in the DD one and
220 an inverse correlation with the milling fraction particle size was observed for both processes.
221 Several studies have been conducted on the distribution of fumonisins in dry-milled maize fractions
222 (Katta et al., 1997; Scudamore & Patel, 2000; Broggi et al., 2002; Brera et al., 2004; Bullerman &
223 Bianchini, 2007; Castells et al., 2008; Pietri et al., 2009; Vanara et al., 2009; Burger et al., 2013;
224 Aprodu & Banu, 2015; Generotti et al., 2015; Bordini et al., 2017; Vanara et al., 2018; Scarpino et
225 al., 2020). Although the approach of each study was different, the cited studies reported a similar
226 trend of the FB distribution in the various maize-milled fractions, in particular with respect to those
227 that considered the 2 different dry-milling processes (DD and TD) at the same time and separately
228 (Vanara et al., 2018; Scarpino et al., 2020).
229 The fumonisin A-series are N-acetyl analogs of FBs and, in 1998, Van der Westhuizen et al.
230 reported that these series of fumonisins, have the ability to inhibit sphingosine N-acyltransferase,
231 just like FBs. FA_1 on average represented about 60% of FA_{TOT}, while FA_2 represented the
232 remaining 40%. Although the FA_{TOT} concentration was about 30 times lower than that of FB_{TOT}, its
233 distribution was almost the same as that of FB_{TOT}. The cleaning phase on average led to a reduction
234 of -54%, compared to the pre-cleaned whole grain. The FA_{TOT} content in the animal feed flours
235 from the DD and TD processes increased by 2.4 and 1.9 times, respectively, compared to the pre-
236 cleaned whole grain, while the FA_{TOT} content in the germ from the DD (-53%) and TD (-57%)

237 processes instead reduced. The maize flour, break meal and pearl meal (DD process) showed an
238 average FA_{TOT} decrease of -61%, -87% and -90%, respectively, compared to the pre-cleaned whole
239 grain, while the small and medium hominy grits and the flaking grits (TD process) showed a
240 decrease of -91%, -95% and -97%, respectively.
241 The redistribution of the other *Fusarium* mycotoxins and fungal metabolites, produced by species
242 belonging to the *Liseola* section together with the FB and FA, in the different maize dry-milling
243 fractions is reported in Table 2 and Table 3. The cleaning phase always led to a similar reduction of
244 the MON (-45%), BEA (-45%), FUS (-45%), FSA (-53%), FnA (-37%), fusarin C (-60%) and BIK
245 (-27%) contents, in comparison to the pre-cleaned whole grain. The feed flour always showed
246 increases in the MON, BEA, FUS, FSA, FnA, fusarin C and BIK contents of 1.6, 3.4, 2.5, 3.4, 2.3,
247 5.3 and 2.8 times, respectively, compared to the pre-cleaned whole grain. The germ from the DD
248 and TD processes instead presented reduced MON (-41%) and BIK (-54%) contents, an unchanged
249 BEA content, but also increases in the FUS, FSA, FnA and fusarin C contents of 1.6, 3.8, 1.3 and
250 1.9 times, respectively, compared to the pre-cleaned whole grain. An inverse correlation between
251 the level of contamination of the food grade milling fraction and the particle size was also observed
252 for these other *Fusarium* mycotoxins and metabolites. The maize flour of the DD process was the
253 fraction with the lowest particle size and the smallest reduction, which on average was equal to -
254 41% for MON, -62% for BEA, -71% for FUS, -69% for FSA, -39% for FnA, -54% for fusarin C
255 and -60% for BIK, compared to the pre-cleaned whole grain. The flaking grits, the fraction with the
256 highest particle size and greatest reduction, on average showed decreases of -87% for MON, -98%
257 for BEA, -94% for FUS, -92% for FSA, -88% for FnA, -60% for fusarin C and -95% for BIK.
258 As far as the toxicological relevance of these other mycotoxins co-produced with FB_{TOT} by the
259 *Fusarium* spp. of the *Liseola* section is concerned, particular attention should be paid to fusarin C.
260 Although IARC classified it as part of the 2B group in 1993, due to its carcinogenic potential for
261 humans, together with FB_1 and FB_2, it has not yet been taken into consideration in any legislation.
262 To date, no regulatory limits have also been established concerning the presence of MON. Jonsson

et al. (2015) reported a high acute toxicity of MON in rats, with the LD_{50} value being at the same level as that of T-2 and HT-2 toxins, the most toxic of the *Fusarium* mycotoxins. Moreover, a recent review (Fremy et al., 2019) has underlined an interactive toxicity of MON and FB_1. For these reasons, EFSA has recently requested the collection of further data on the presence of MON in food and feeds to allow a comprehensive human risk assessment to be made (EFSA, 2018).

Toxic effects have also been documented for FSA (Dhani et al., 2017; Mamur et al., 2018), BEA (Ojcius et al., 1991; Logrieco et al., 2002) and for FUS (Logrieco et al., 1996; Ritieni et al., 1997) in humans and animals. FnA is closely related to FSA and is enzymatically derived from it (Fumero et al., 2020), but its toxicity towards humans and animals has not been evaluated extensively. Similarly, there is also a lack of toxicological data for BIK and further support studies are certainly needed (Santos et al., 2020).

DON was the main regulated mycotoxin among the fungal metabolites produced by *Fusarium* spp. of the Discolor section. However, together with DON, its plant metabolites, that is, DON-3-G, 3-ADON and 15-DON, were always detected in all the maize fractions of both the dry-milling processes. The fate of DON_{TOT} in the milling fractions is reported in Figure 4. The relative abundance, compared to DON_{TOT}, was 56% for DON, 29% for DON-3-G, 14% for 3-ADON and 1% for 15-ADON. Interestingly, the DON and the DON-3-G percentages in DON_{TOT} varied as a function of the different milling fractions, as highlighted by the DON-3-G/DON molar ratio (Figure 5). This ratio increased significantly, compared to the pre-cleaned whole grain, in both the DD (+44%) and TD (+33%) germs and, albeit to a lesser extent, in the break meal (+40%) and pearl meal (+36%) from the DD process and in the small hominy grits (+21%), medium hominy grits (+16%) and flaking grits (+30%) from the TD process. On the other hand, the DON-3-G/DON molar ratio decreased in the animal feed flour (-23% and -37% for DD and TD, respectively) and maize flour (-12%). The higher content of this masked mycotoxin, which is not usually monitored, in certain products and by-products, highlights an even greater risk of the consumption of the derived food. This important aspect for consumer health has never been reported before.

289 The cleaning step on average reduced the DON_{TOT} content, in comparison to that of the pre-cleaned
290 whole grain content, by -35%. Overall, the germ and the animal feed flour from both processes
291 represented the fractions with the highest DON_{TOT} content. The animal feed flour on average
292 increased DON_{TOT} by 2.1 times, in comparison to the pre-cleaned whole grain. On the other hand,
293 contrary to what has been recorded for most metabolites produced by *Fusarium* spp. of the *Liseola*
294 section, the DON_{TOT} content always significantly increased in the germ, for both the DD and TD, in
295 comparison to the post-cleaned wholegrain, by 2.8 times. As for the endosperm products, the maize
296 flour, break meal and pearl meal (DD process) showed DON_{TOT} decreases, in comparison to the
297 pre-cleaned whole grain, of -61%, -71% and -78%, respectively, while the small and medium
298 hominy grits and the flaking grits (TD process) showed decreases of -76%, -83% and -93%,
299 respectively, thus confirming an inverse relationship with the particle size.
300 ZEA, another regulated mycotoxin produced by *Fusarium* spp. of the *Discolor* section, co-occurred
301 in all the maize fractions with the masked or modified forms ZEA-S, α-ZEA-ol and β-ZEA-ol. ZEA
302 accounted for about 27% of ZEA_{TOT}, ZEA-S for 60%, α-ZEA-ol for 5% and β-ZEA-ol for 8%. The
303 redistribution of ZEA_{TOT} in the dry-milling fractions is shown in Figure 6. The cleaning phase on
304 average led to a reduction of -60%, compared to the ZEA_{TOT} content of the pre-cleaned whole
305 grain. The animal feed flour from both the DD and TD processes on average presented a 2.8 times
306 increase of the ZEA_{TOT} content, compared to the pre-cleaned whole grain. As for the germ, the
307 ZEA_{TOT} content of both DD and TD showed a variable redistribution over the years and on average
308 increased 1.6 times, compared to the pre-cleaned whole grain, and 5.2 times, compared to the post-
309 cleaned whole grain. The endosperm fractions for human consumption, that is, the maize flour,
310 break meal and pearl meal (DD process), showed ZEA_{TOT} decreases, compared to the pre-cleaned
311 whole grain, of 48%, 81% and 85%, respectively, while the small and medium hominy grits and the
312 flaking grits (TD process) showed decreases of 89%, 89% and 94%, respectively.
313 The distribution of DON and ZEA in the maize dry-milled fractions has only been reported in a few
314 studies (Schaafsma et al., 2004; Brera et al., 2006; Schollenberger et al., 2008; Burger et al., 2013),

315 and some of these only considered fractions purchased in local markets (Yang et al., 2019) and
316 which were not derived from the same milling process. Moreover, most of the scientific literature
317 has focused on wheat milling and its derived fractions (Scudamore et al., 2009; Kostelanska et al.,
318 2011; Schwake-Anduschus et al., 2015; Edwards et al., 2018; Khaneghah et al., 2018; Guo et al.,
319 2020).
320 Like us, Brera et al. (2006) reported that the ZEA level was higher in bran and high fat fractions,
321 such as germs. The present data are also in accordance with the redistribution described by
322 Schaafsma et al. (2004), Schollenberger et al. (2008) and Burger et al. (2013). As for DON, the
323 effects of the process may vary according to the degree of fungal penetration of the endosperm: if
324 the fungal penetration is limited, a notable reduction in the DON level in maize fractions intended
325 for human consumption can be achieved (Brera et al 2006; Khaneghah et al., 2018).
326 The present data have pointed out the presence, together with DON and ZEA, of their associated
327 metabolites (masked or modified). DON-3-G and ZEA-S are phase II plant metabolites of the
328 *Fusarium* mycotoxins DON and ZEA, respectively (Berthiller et al., 2013). These associated forms
329 could be hydrolysed in the digestive tract of mammals, thereby contributing to the total dietary
330 exposure of individuals to DON (Berthiller et al., 2011). On the other hand, the acetylated
331 derivatives of DON, that is, 3-ADON and 15-ADON, are usually considered as derived metabolites
332 of phase I (Pinton et al., 2012). Moreover, 3-ADON has been found to be less toxic than DON,
333 while 15-ADON presents a higher toxicity than its precursor DON, while α-ZEAol and β-ZEAol
334 are phase I plant metabolites of ZEA, with a higher toxicity level and greater hyperestrogenic
335 effects, especially for α-ZEAol (Berthiller et al., 2013). Thus, all these modified forms should be
336 considered as additional contributing factors of the total dietary exposure to DON and ZEA and
337 should also be taken into account for correct risk assessments and food safety (JECFA, 2010; CAC,
338 2011; Lorenz et al., 2019).
339 The fate of the $CULM_{TOT}$, fungal metabolites produced by *Fusarium* spp. of the *Discolor* section, is
340 shown in Figure 7. CULM accounted for about the 38% of $CULM_{TOT}$, 5-OH-CULM for the 30%,

15-OH-CULM for the 24% and 15-OH-culmoron for the 9%. The cleaning phase led to an average reduction of -34%, compared to the $CULM_{TOT}$ content of the pre-cleaned whole grain. The animal feed flour from both the DD and TD processes on average increased 2.5 times, compared to the pre-cleaned whole grain. Like the DON_{TOT} redistribution, the $CULM_{TOT}$ content always significantly increased in the germ, for both the DD and TD processes, compared to the content in the post-cleaned wholegrain, that is, on average by 3.2 times. When considering the maize fractions destined for human consumption with the smallest and largest particle sizes, the maize flour and the flaking grits on average showed $CULM_{TOT}$ decreases, compared to the pre-cleaned whole grain, of -64% and -90%, respectively.

The fate of other fungal metabolites produced by *Fusarium* spp. of the *Discolor* and *Roseum* sections, including AUR, BUT and EQU, is summarised in Table 4. The cleaning phase generally led to a notable reduction of the AUR content (-60%), compared to the pre-cleaned whole grain, but a slight increase was recorded for BUT and EQU of +2% and +7%, respectively. Overall, the animal feed flour from both the DD and TD processes always showed increases of the AUR, BUT and EQU contents of 2.2, 2.5 and 4.1 times, respectively, compared to the pre-cleaned whole grain. On the other hand, the germ only presented a reduction of the AUR (-46%) and EQU (-54%) contents, but a 1.4 times increase in the BUT content, compared to the pre-cleaned whole grain. Like the other fungal metabolites, among the endosperm fraction intended for human consumption, maize flour on average showed a decrease for the AUR (-85) and BUT (-20%) contents, while the EQU content increased (+37%), compared to the pre-cleaned whole grain. The flaking grits always showed a reduction of the AUR (-99%), BUT (-90%) and EQU (-96%) contents.

Although CULM was previously reported to have a limited toxic potential in mammals (Dowd et al., 1989; Miller & MacKenzie, 2000), Woelfingseder et al. (2019) have recently reported that CULM could partially inhibit the glucuronidation activity of human liver microsomes. The study carried out by Woelfingseder et al. (2019) underlined the necessity of further studies on the relevance of CULM as a potentially co-occurring modulator of DON toxicokinetics in vivo, and it

367 led to the discussion about the possibility of classifying CULM not only as a secondary fungal
368 metabolite but also as an "emerging mycotoxin".
369 AUR is a golden yellow *F. graminearum* polyketide bioactive pigment produced under plant stress
370 conditions (Medentsev et al., 2005). It is considered a neglected mycotoxin (Streit et al., 2013;
371 Jarolim et al., 2018), since it is known to induce oxidative stress, cytotoxicity and genotoxicity in
372 human colon cells (Jarolim et al., 2018) and also shows toxicity for differentiated intestinal porcine
373 epithelial cells (IPEC-J2) when combined with DON (Springler et al., 2016). BUT possesses the
374 potential to induce myocardial toxicity (Liu et al., 2007), while EQU has recently been reported to
375 be toxic for chicks (Tayo et al., 2017).
376 Among all the previous described emerging *Fusarium* mycotoxins and fungal metabolites, only the
377 fate of MON has been considered in the scientific literature, through the dry-milling of maize
378 (Scarpino et al., 2020), while the other ones have never been reported before in maize dry-milled
379 fractions. Moreover, to the best of the authors' knowledge, this is the first time that the presence and
380 distribution of DON and ZEA have been reported in dry-milled fractions together with their main
381 masked or modified metabolites. Schollenberger et al. (2008) only reported 3-ADON and 15-
382 ADON for DON, and α-ZEAol and β-ZEAol for ZEA, but did not consider DON-3-G or ZEA-S,
383 which are the most commonly and abundantly modified forms of DON and ZEA in food and feeds.
384 Considering the mycotoxins produced from fungal species that do not belong to the *Fusarium*
385 genus, the highest AF_{TOT} contamination levels were present in the milling fractions during the year
386 2012 (Figure 8), followed by the year 2014, while the levels were between the limit of detection
387 (LOD) and the limit of quantification (LOQ) for 2013. AFB_1 was the form that was present the
388 most, and on average represented about the 70% of the AF_{TOT} content. The fraction with the highest
389 contamination level was the germ of the DD process, in both 2012 and 2014, with a significant
390 increase of 13.3 times in 2012 and a lower increase, that is, of 2.3 times, in 2014, compared to the
391 pre-cleaned whole grain. Moreover, the germ from the TD process presented a significantly lower
392 AF_{TOT} contamination in 2012 than the DD germ. The maize dry-milling products with a

393 significantly lower content in 2012 were the pearl meal of the DD process and the small hominy
394 grits of the TD process, with an average AF_{TOT} content reduction of 60% for both fractions,
395 compared to the pre-cleaned whole grain. On the other hand, no significant differences were
396 recorded for any of the fractions in any of the lots for 2013 and 2014. However, it is important to
397 highlight that since AF_{TOT} was present at low contamination levels and since fungal growth often
398 occurs in localised hot spots, the mycotoxin distribution in contaminated lots tends to be very
399 heterogeneous and the sampling has even more effect on these mycotoxins (Streit et al., 2012).
400 The redistribution of aflatoxins in dry-milled maize fractions was previously considered by Brera et
401 al. (2006), Castells et al. (2008) and Pietri et al. (2009). According to these studies, aflatoxin
402 contamination was uniformly distributed and was more superficial and concentrated in the germ
403 than fumonisin contamination, which conversely affected the inner layers of the kernels and was
404 mainly concentrated in the finer size fractions. However, to the best of the authors' knowledge,
405 among the regulated mycotoxins, the AFs, as well as DON and ZEA distribution in maize-milled
406 fractions, have never been treated before at the same time and separately on the same maize lots
407 through the comparison of 2 different dry-milling processes (DD and TD).
408 Some metabolites, such as ENNs, T-2 and HT-2 Toxin, NIV, DAS and *Alternaria* metabolites were
409 present at detectable levels in only a few samples of the pre-cleaned grain. For this reason, their
410 distribution was not evaluated.
411 Table 5 summarises the decontamination of the different detected mycotoxins and fungal
412 metabolites in the endosperm fractions (the sum of the maize flour, break meal and pearl meal from
413 DD and the sum of small and medium hominy grits and flaking grits from TD) obtained from
414 different milling processes. Overall, the endosperm fractions from the TD process resulted in less
415 contamination than DD. Thus, considering the inverse relationship with the particle size, flaking
416 grits represented the healthiest maize product for all the metabolites, while the abatement was
417 always lower for maize flour. Taking FB_{TOT} and DON_{TOT} as references, FnA, fusarin C, MON,
418 BUT and AF_{TOT} resulted in an overall higher contamination of the endosperm fractions.

Nevertheless, it should be considered that very variable behavior was recorded for the fusarin C and AF_{TOT} (data not shown), due to their low levels of contamination. On the other hand, FA_{TOT}, BIK, BEA, FUS, ZEA_{TOT} and AUR showed a higher decontamination in both processes, while FSA, CULM and EQU resulted in a similar behaviour to FB_{TOT} and DON_{TOT}.

The greater permanence of some mycotoxins and fungal metabolites in the maize food-grade products from the TD and DD dry-milling processes points out a higher risk of exposure for the end consumers. The different fate of the contaminants observed in the present work could allow regulation limits to be defined considering the health impact of the aforementioned mycotoxins.

4. Conclusions

This is the first time that the redistribution and co-occurrence of a broad spectrum of mycotoxins and fungal metabolites have been considered and reported in an industrial dry-milling study, through the application of different degermination processes.

The obtained data confirm that a cleaning process is essential to reduce the risk of contamination of almost all the mycotoxins and fungal metabolites. Moreover, the endosperm fractions from the TD process generally showed a lower contamination than DD, for all the metabolites, and an inverse relationship with particle size was always detected.

However, the weaker decontamination of some mycotoxins and fungal metabolites (AF_{TOT}, DON-3-G, FnA, fusarin C, MON and BUT) in the food-grade milling fractions points to a higher risk of exposure for the end consumers, particularly when environmental conditions favour their simultaneous increase in whole grain at harvesting. It is also of great importance to point out the concentrations of some mycotoxins and fungal metabolites that were found in the germ (AF_{TOT} and kojic acid, DON_{TOT}, $CULM_{TOT}$, BUT, FUS, FSA, FnA and in some cases ZEA_{TOT} and fusarin C), as well as the significant increase in the content of almost all the mycotoxins and fungal metabolites in the animal feed flour, with a consequent negative impact on animal health.

444 The co-occurrence of a such a high number of mycotoxins and fungal metabolites and their
445 different fates during the dry-milling process should be considered in future risk assessment studies
446 to correctly assess the risk to exposure. Moreover, continuous exposure to these mycotoxins and
447 fungal metabolites should not be underestimated for consumers in many parts of the world where
448 maize is a staple food, or where it is used for the baby food supply chain and for the celiac
449 population in developed countries, due to the high consumption of maize gluten-free products.
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Figure Captions

Figure 1. Distribution of the particle sizes of the maize products after the milling process.

Figure 2. Total fumonisin B (FB_{TOT}) distribution in the fractions of different dry milling processes and different maize lots.

Figure 3. Total fumonisin A (FA_{TOT}) distribution in the fractions of different dry milling processes and different maize lots.

Figure 4. Total deoxynivalenol (DON_{TOT}) distribution in the fractions of different dry milling processes and different maize lots.

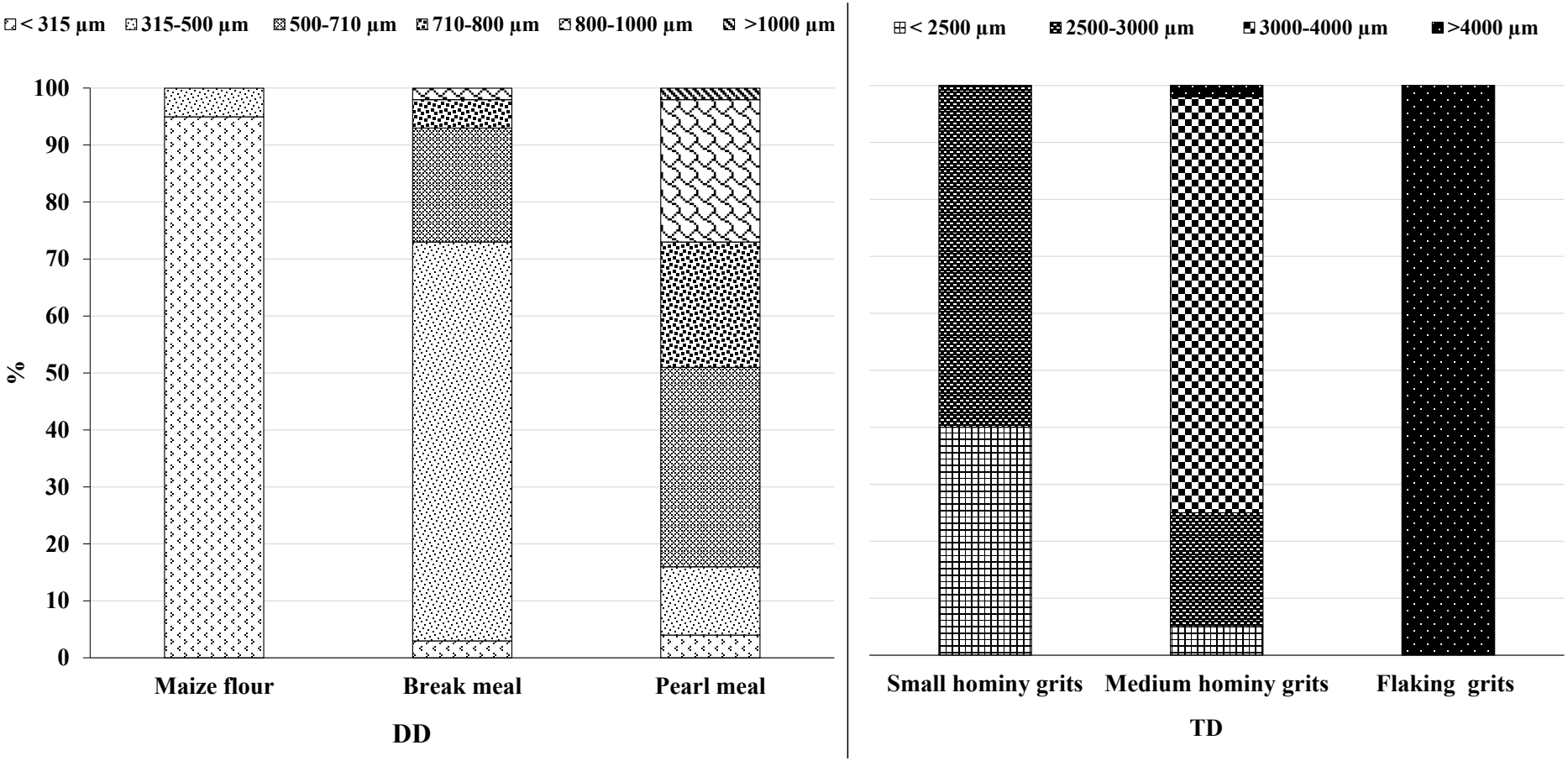
Figure 5. Averaged DON-3-G/DON molar ratio distribution in the fractions of different dry milling processes.

Figure 6. Total zearalenone (ZEA_{TOT}) distribution in the fractions of different dry milling processes and different maize lots.

Figure 7. Total culmorin ($CULM_{TOT}$) distribution in the fractions of different dry milling processes and different maize lots.

Figure 8. Total aflatoxin (AF_{TOT}) distribution in the fractions of different dry milling processes and maize lots.

733 **Figure 1.**



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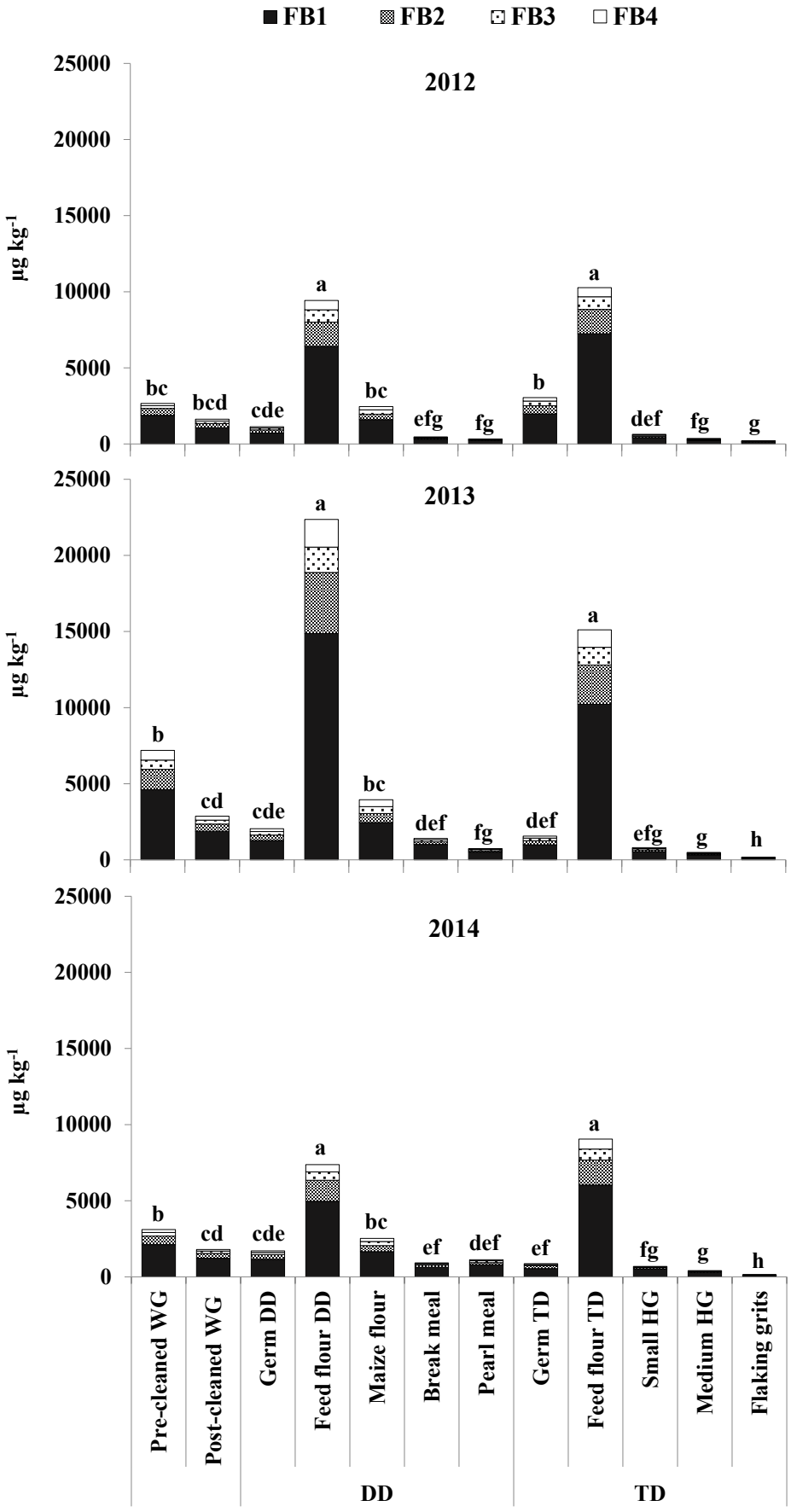
736 **Figure 2.**



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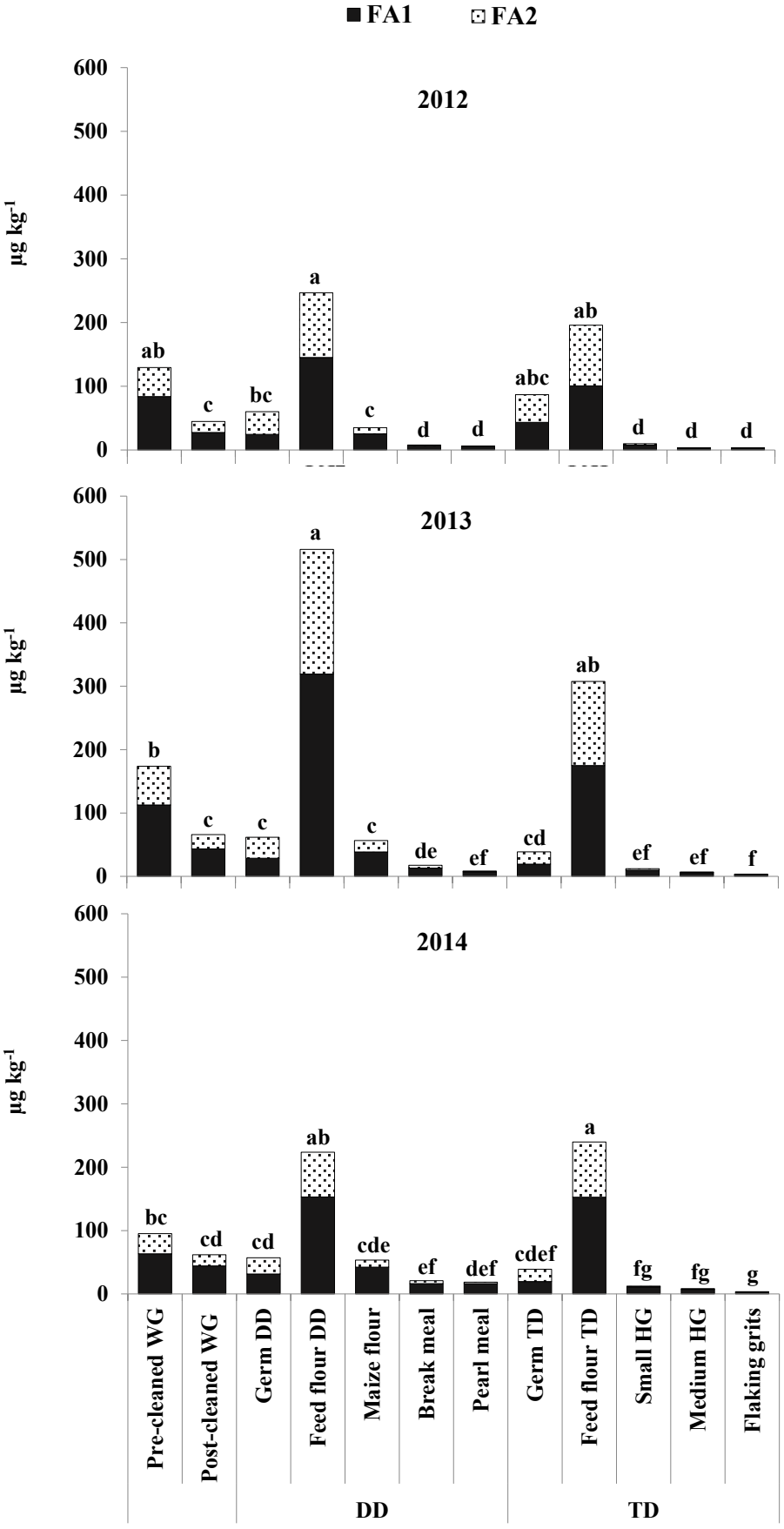
738 DD = dry-degermination; TD = tempering-degermination; WG = whole grain; HG = hominy grits.
739 Different letters above the bars indicate significant differences in the fractions ($p < 0.05$).

740 **Figure 3.**



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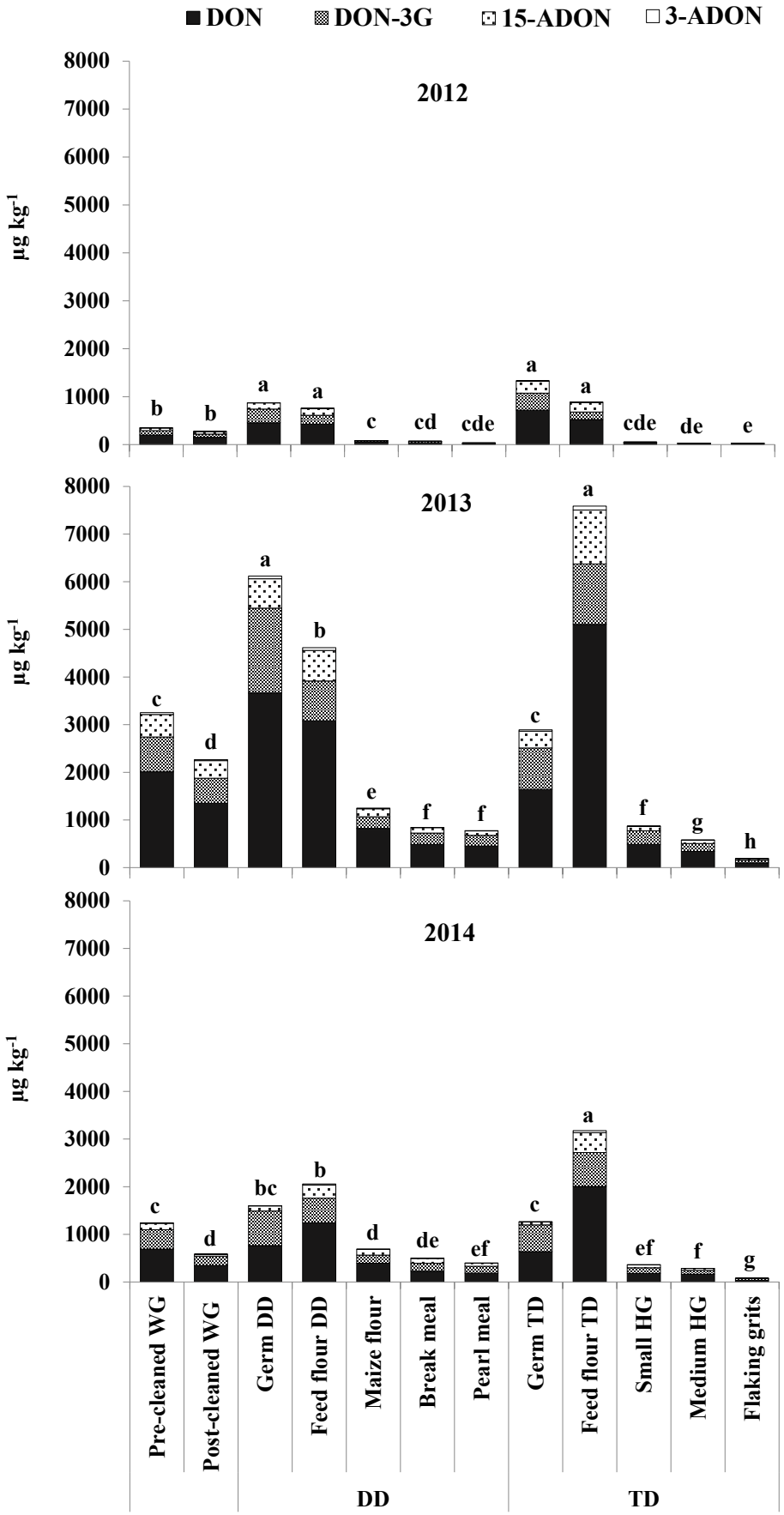
742 DD = dry-degermination; TD = tempering-degermination; WG = whole grain; HG = hominy grits.
743 Different letters above the bars indicate significant differences in the fractions ($p < 0.05$).

744 **Figure 4.**



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746 DD = dry-degermination; TD = tempering-degermination; WG = whole grain; HG = hominy grits.
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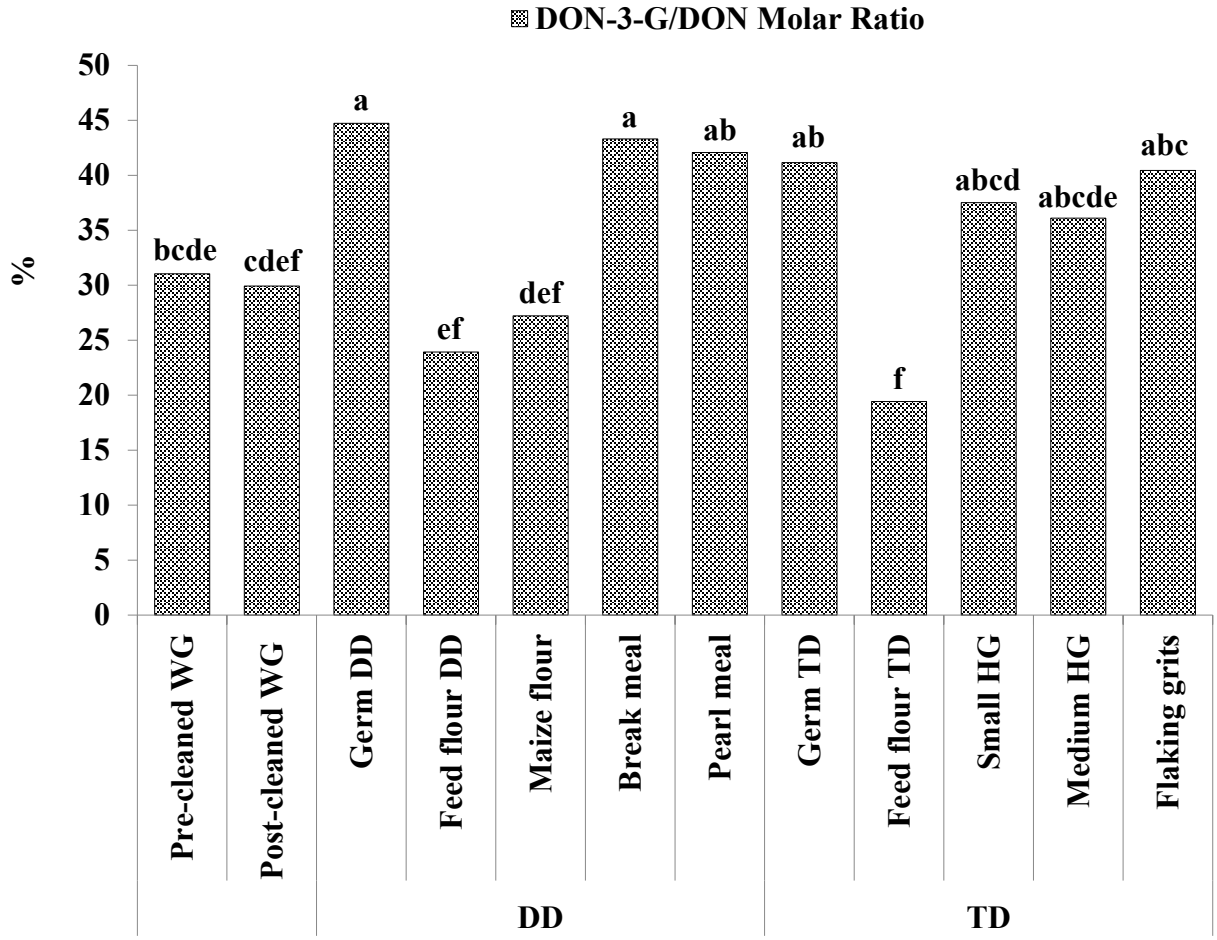
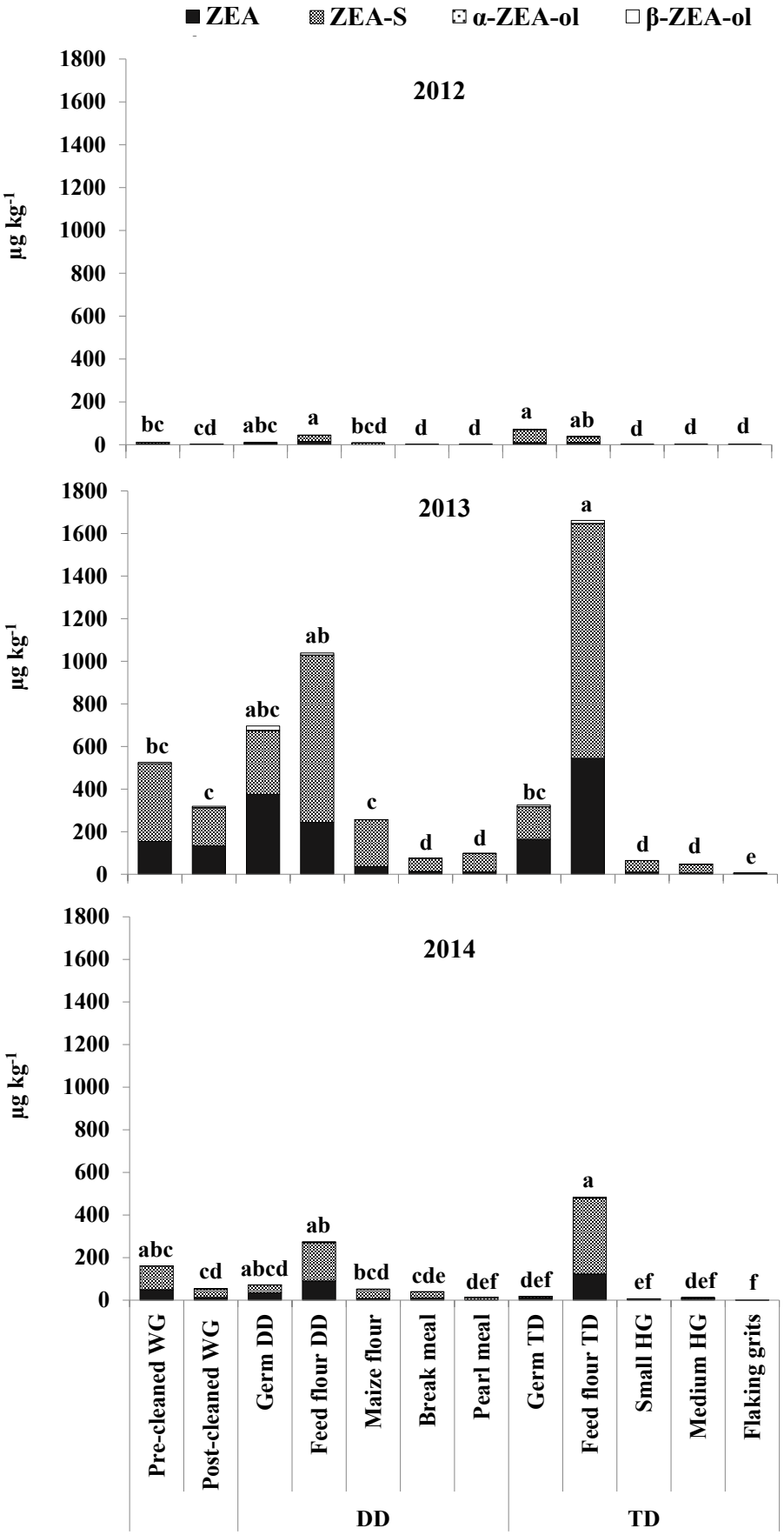
Figure 5.



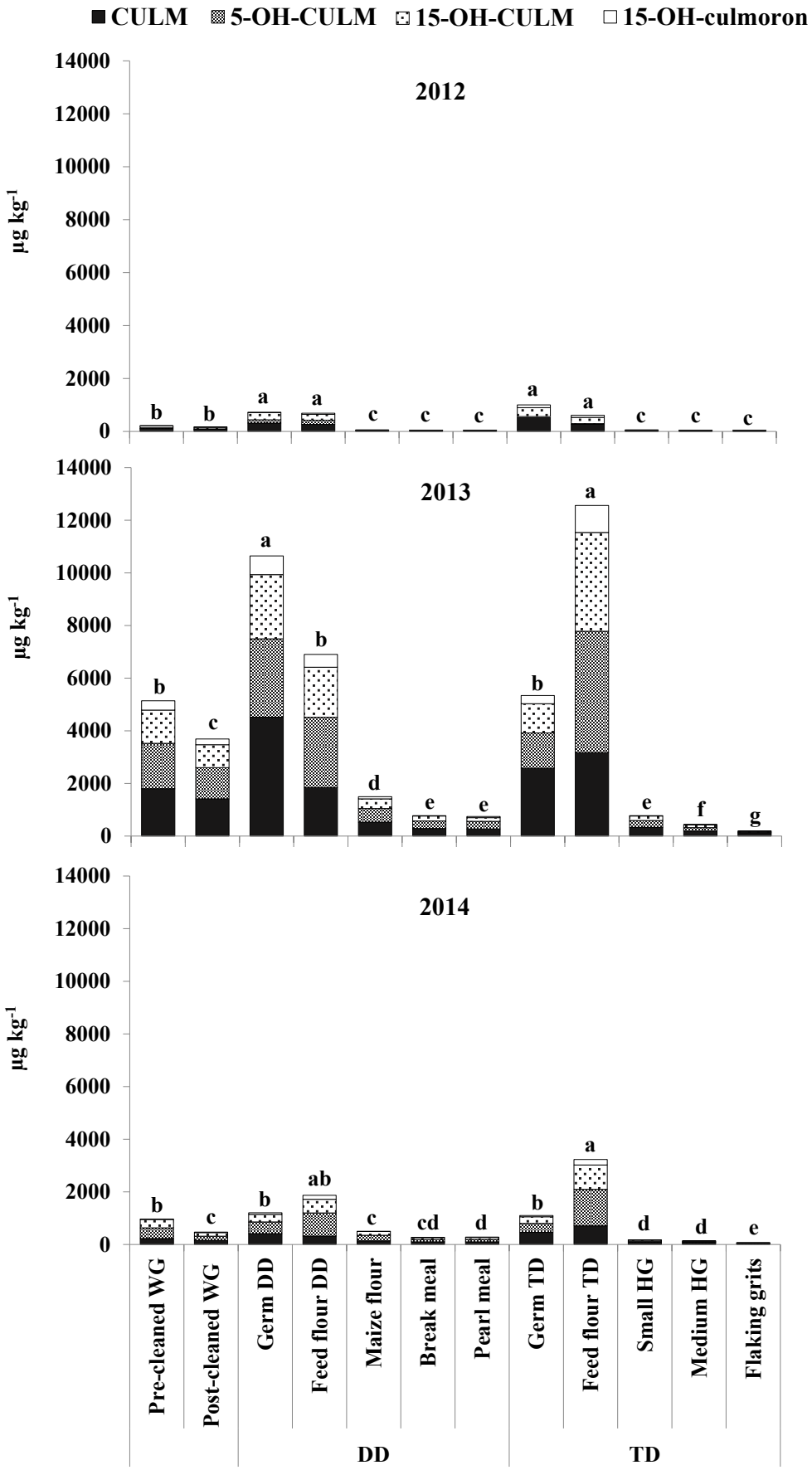
DD = dry-degermination; TD = tempering-degermination; WG = whole grain; HG = hominy grits. Different letters above the bars indicate significant differences in the fractions ($p < 0.05$).

Figure 6.



DD = dry-degermination; TD = tempering-degermination; WG = whole grain; HG = hominy grits. Different letters above the bars indicate significant differences in the fractions ($p < 0.05$).

771 **Figure 7.**



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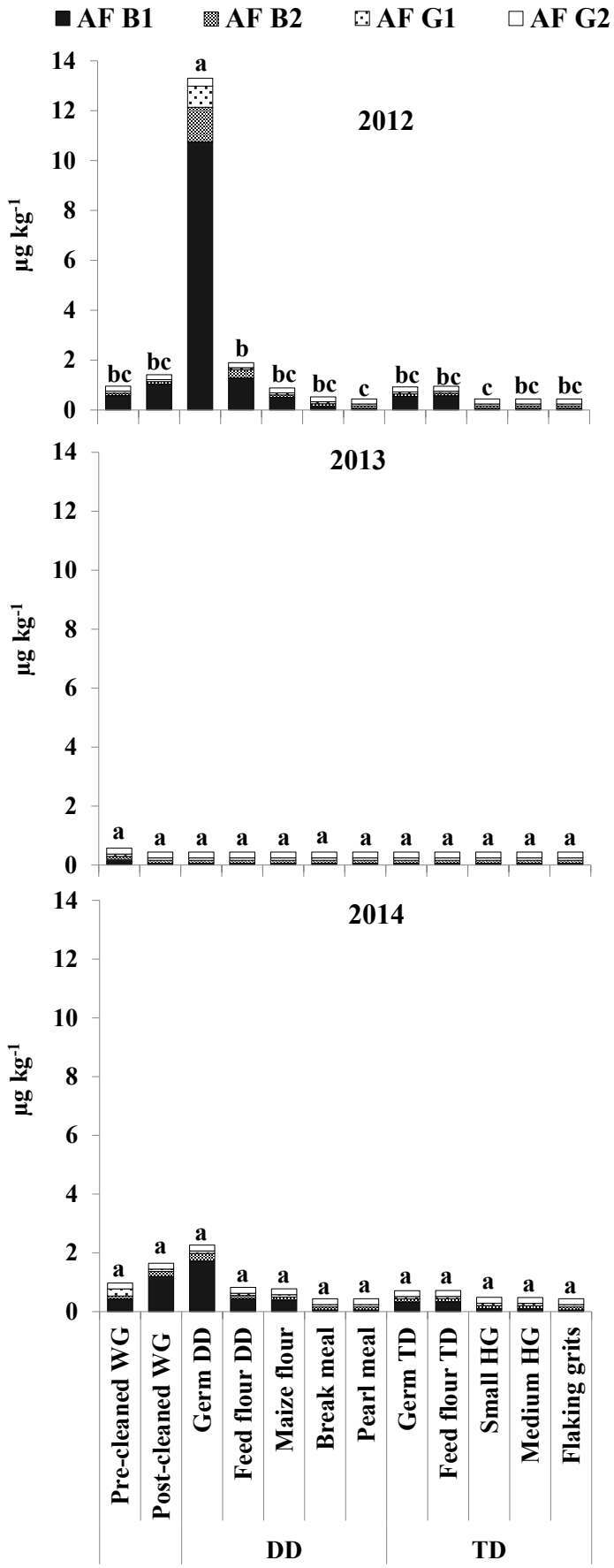
773 DD = dry-degermination; TD = tempering-degermination; WG = whole grain; HG = hominy grits.
774 Different letters above the bars indicate significant differences in the fractions ($p < 0.05$).

775 **Figure 8.**



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777 DD = dry-degermination; TD = tempering-degermination; WG = whole grain; HG = hominy grits.
778 Different letters above the bars indicate significant differences in the fractions ($p < 0.05$).

Tables

Table 1. Maize lots processed in the industrial mill, ranked according to the maize production year and the average values of the main regulated, masked, emerging mycotoxins and other secondary fungal metabolites in pre-cleaned whole grain expressed as μg kg^{-1} ± standard deviation (SD).

Main Fungal Producers	Mycotoxin or Fungal Metabolite	Year / Hybrid		
		2012	2013	2014
		Pioneer P1547	Pioneer P1547	Pioneer P0722
Fusarium verticillioides *F. proliferatum* *F. temperatum*	FB_{TOT} ± SD	2664 ± 459	7190 ± 1971	3107 ± 308
	FA_{TOT} ± SD	129 ± 61	174 ± 56	95 ± 41
	HFB_1 ± SD	< LOQ[a]	3.4 ± 2.6	< LOQ
	FSA ± SD	39 ± 62	399 ± 83	269 ± 47
	FnA ± SD	494 ± 131	939 ± 338	747 ± 65
	Fusarin C ± SD	< LOQ	107 ± 41	15 ± 24
	BIK ± SD	76 ± 47	112 ± 13	79 ± 6
Fusarium proliferatum *F. temperatum* *F. subglutinans*	MON ± SD	351 ± 273	373 ± 38	357 ± 58
	BEA ± SD	28 ± 10	111 ± 52	43 ± 4
	FUS ± SD	313 ± 152	413 ± 137	247 ± 118
	ENN_{TOT} ± SD	< LOQ	< LOQ	< LOQ
Fusarium graminearum *F. culmorum*	DON_{TOT} ± SD	350 ± 134	3254 ± 320	1243 ± 152
	ZEA_{TOT} ± SD	12 ± 7	526 ± 181	162 ± 63
	$CULM_{TOT}$ ± SD	221 ± 61	5140 ± 490	971 ± 142
	AUR ± SD	463 ± 359	3593 ± 608	989 ± 311
	BUT ± SD	132 ± 35	901 ± 199	95 ± 8
	DAS ± SD	0.9 ± 1.0	0.5 ± 0.3	< LOQ
	NIV ± SD	< LOQ	15 ± 3	5 ± 10
Fusarium langsethiae, F. poae *F. sporotrichioides*	T-2 toxin ± SD	1.5 ± 1.5	2.0 ± 1.4	1.9 ± 2.0
	HT-2 toxin ± SD	< LOQ	< LOQ	< LOQ
Fusarium equiseti	EQU ± SD	45 ± 62	22 ± 13	9 ± 8
Aspergillus spp.	AF_{TOT} ± SD	1.0 ± 0.5	0.6 ± 0.3	1.0 ± 0.6
	Kojic acid	289 ± 132	106 ± 126	1338 ± 264
Alternaria spp.	AOH	< LOQ	< LOQ	< LOQ
	AME	< LOQ	< LOQ	< LOQ
	TEN	< LOQ	< LOQ	< LOQ
	TeA	6.2 ± 4.4	< LOQ	< LOQ
	ALS	1.0 ± 1.5	0.4 ± 0.5	< LOQ

The reported contamination means for each lot were based on 2 repetitions.

[a]LOQ = limit of quantification = 1.6 μg kg^{-1} for HFB_1; 4.8 μg kg^{-1} for fusarin C; 0.1 μg kg^{-1} for ENN_{TOT}; 0.4 μg kg^{-1} for DAS; 1.2 μg kg^{-1} for NIV; 3.2 μg kg^{-1} for HT-2 toxin; 0.4 μg kg^{-1} for AOH; 0.032 μg kg^{-1} for AME; 0.08 μg kg^{-1} for TEN; 8.0 μg kg^{-1} for TeA; 0.4 μg kg^{-1} for ALS.

Table 2. Moniliformin (MON), beauvericin (BEA) and fusaproliferin (FUS) distributions in the fractions of different dry milling processes and different maize lots.

Dry milling process[a]	Milling fraction[b]	MON (µg kg^{-1})			BEA (µg kg^{-1})			FUS (µg kg^{-1})		
		2012	2013	2014	2012	2013	2014	2012	2013	2014
DD	Pre-cleaned WG	351 abc	373 ab	357 bc	28 bcd	111 bc	43 abc	313 ab	413 abc	247 ab
	Post-cleaned WG	155 bcd	186 bc	249 cde	13 def	36 de	38 bc	125 bc	207 bcd	182 ab
	Germ	109 cd	228 abc	324 bcd	22 bcde	61 cd	76 ab	345 ab	841 a	338 ab
	Feed four	536 ab	491 a	591 ab	150 a	302 ab	127 a	737 a	1216 a	552 ab
	Maize flour	211 abcd	182 bc	246 cde	13 cdef	20 def	22 bcd	50 cd	127 cde	103 bc
	Break meal	105 cd	98 c	145 ef	4 efg	5 fgh	14 cde	< LOQ[c] d	69 de	< LOQ c
	Pearl meal	98 cd	140 bc	106 f	1 g	2 gh	9 cde	< LOQ d	< LOQ e	< LOQ c
TD	Germ	266 abc	170 bc	178 def	66 abc	29 de	15 bcde	702 a	779 ab	187 ab
	Feed four	580 a	635 a	676 a	94 ab	314 a	139 a	649 a	1109 a	715 a
	Small HG	117 cd	172 bc	170 def	3 fg	9 efg	4 def	< LOQ d	< LOQ e	< LOQ c
	Medium HG	83 cd	125 bc	145 ef	1.0 g	3 gh	2 ef	< LOQ d	< LOQ e	< LOQ c
	Flaking grits	53 d	27 d	54 g	0.8 g	1.1 h	0.7 f	< LOQ d	< LOQ e	< LOQ c
	p-value	0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

[a]dry milling process: DD, dry-degermination; TD, tempering-degermination.
[b]WG = whole grain; HG = hominy grits.
[c]LOQ = limit of quantification = 0.008 µg kg^{-1} for BEA; 40 µg kg^{-1} for FUS.
Means followed by different letters are significantly different (the significance level is shown in the table).
The reported mycotoxin contamination values for the fractions of each lot/year are based on 2 repetitions.

Table 3. Fusaric acid (FSA), fusarinolic acid (FnA), Fusarin C and bikaverin (BIK) distributions in the fractions of different dry milling processes and different maize lots.

Dry milling process[a]	Milling fraction[b]	FSA (µg kg^{-1})						FnA (µg kg^{-1})						Fusarin C (µg kg^{-1})						BIK (µg kg^{-1})					
		2012		2013		2014		2012		2013		2014		2012		2013		2014		2012		2013		2014	
	Pre-cleaned WG	39	b	399	b	269	a	494	b	938	b	747	b	< LOQ	a	107	b	14	a	76	b	112	b	79	b
	Post-cleaned WG	< LOQ[c]	b	244	c	163	ab	317	bcd	567	bcd	473	cd	< LOQ	a	< LOQ	b	< LOQ	a	61	bc	71	c	59	bc
DD	Germ	147	ab	785	a	520	a	421	bc	795	bc	1094	ab	24	a	< LOQ	b	< LOQ	a	37	bc	45	cd	54	bc
	Feed four	241	a	1135	a	352	a	1425	a	2732	a	1201	a	39	a	219	a	< LOQ	a	212	a	316	a	181	a
	Maize flour	< LOQ	b	197	c	65	bcd	356	bcd	572	bcd	371	de	< LOQ	a	22	b	< LOQ	a	32	c	41	cd	33	bc
	Break meal	< LOQ	b	193	c	103	abc	176	bcde	380	cde	218	ef	< LOQ	a	< LOQ	b	< LOQ	a	< LOQ	d	14	e	8	d
	Pearl meal	< LOQ	b	159	c	< LOQ	d	155	cde	378	cde	284	def	< LOQ	a	< LOQ	b	< LOQ	a	< LOQ	d	< LOQ	f	8	d
TD	Germ	465	a	616	ab	432	a	1288	a	781	bc	683	bc	< LOQ	a	< LOQ	b	< LOQ	a	48	bc	31	d	27	c
	Feed four	247	a	1036	a	390	a	1337	a	2008	a	1270	a	26	a	52	b	34	a	249	a	278	a	227	a
	Small HG	< LOQ	b	< LOQ	d	33	cd	212	bcde	295	de	250	ef	< LOQ	a	< LOQ	b	< LOQ	a	< LOQ	d	< LOQ	f	< LOQ	d
	Medium HG	< LOQ	b	< LOQ	d	< LOQ	d	128	de	203	e	169	f	< LOQ	a	< LOQ	b	< LOQ	a	< LOQ	d	< LOQ	f	< LOQ	d
	Flaking grits	< LOQ	b	< LOQ	d	< LOQ	d	74	e	85	f	97	g	< LOQ	a	< LOQ	b	< LOQ	a	< LOQ	d	< LOQ	f	< LOQ	d
	p-value	< 0.001		< 0.001		< 0.001		< 0.001		< 0.001		< 0.001		0.383		0.001		0.619		< 0.001		< 0.001		< 0.001	

[a]dry milling process: DD, dry-degermination; TD, tempering-degermination.
[b]WG = whole grain; HG = hominy grits.
[c]LOQ = limit of quantification = 16 µg kg^{-1} for FSA; 4.8 µg kg^{-1} for fusarin C; 8 µg kg^{-1} for BIK.
Means followed by different letters are significantly different (the significance level is shown in the table).
The reported mycotoxin contamination values for the fractions of each lot/year are based on 2 repetitions.

Table 4. Aurofusarin (AUR), butenolide (BUT) and equisetin (EQU) distributions in the fractions of different dry milling processes and different maize lots.

Dry milling process[a]	Milling fraction[b]	AUR (µg kg^{-1})			BUT (µg kg^{-1})			EQU (µg kg^{-1})		
		2012	2013	2014	2012	2013	2014	2012	2013	2014
	Pre-cleaned WG	463 a	3593 b	990 abc	132 abc	901 b	95 bc	45 abcd	22 b	9 bcde
	Post-cleaned WG	91 bc	2155 c	415 cde	192 ab	846 b	63 c	42 abc	16 bc	14 abcd
DD	Germ	371 a	2771 bc	589 bcd	166 abc	771 bc	144 b	31 abc	15 bcd	1.9 cde
	Feed four	777 a	7138 a	2334 ab	391 a	1609 a	186 ab	102 a	105 a	54 a
	Maize flour	27 cd	592 e	222 def	31 bcd	675 bc	134 bc	24 abc	24 ab	22 abc
	Break meal	7 de	139 f	206 defg	28 cd	560 bc	126 bc	6 abcd	3 cd	1.8 de
	Pearl meal	9 de	92 fg	92 fgh	< LOQ d	489 cd	104 bc	2 cd	2 d	1.8 de
TD	Germ	317 ab	984 d	110 efg	426 a	489 cd	123 bc	24 abc	9 bcd	2.0 cde
	Feed four	610 a	10583 a	2970 a	353 ab	1532 a	390 a	88 ab	109 a	39 ab
	Small HG	5 de	142 f	31 gh	11 d	301 e	116 bc	5 abcd	6 bcd	1.2 de
	Medium HG	< LOQ[c] e	65 g	22 h	19 d	318 de	68 bc	3 bcd	4 bcd	1.5 de
	Flaking grits	< LOQ e	60 g	17 h	15 d	128 f	< LOQ d	< LOQ d	2 bcd	0.3 e
	p-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.002	< 0.001	< 0.001

[a]dry milling process: DD, dry-degermination; TD, tempering-degermination.
[b]WG = whole grain; HG = hominy grits.
[c]LOQ = limit of quantification = 2.4 µg kg^{-1} for AUR; 5.6 µg kg^{-1} for BUT; 0.24 µg kg^{-1} for EQU.
Means followed by different letters are significantly different (the significance level is shown in the table).
The reported mycotoxin contamination values for the fractions of each lot/year are based on 2 repetitions.

Table 5. Contamination percentage of different mycotoxins in endosperm fractions obtained from different milling processes.

Main Fungal Producers	Mycotoxin or Fungal Metabolite	Endosperm fraction contamination[a] (%)	
		DD[b]	TD
Fusarium verticillioides *F. proliferatum* *F. temperatum*	FB_{TOT}	14	5
	FA_{TOT}	7	2
	FSA	15	5
	FnA	21	11
	BIK	6	3
Fusarium proliferatum *F. temperatum* *F. subglutinans*	MON	19	13
	BEA	8	2
	FUS	5	4
Fusarium graminearum *F. culmorum*	DON_{TOT}	14	7
	ZEA_{TOT}	11	5
	$CULM_{TOT}$	13	7
	AUR	4	1
	BUT	35	14
Fusarium equiseti	EQU	13	5

[a]Data were calculated according to the balance mass criteria, considering the specific milling yield and the contamination of each fraction.

[b]The occurrence of each mycotoxin is reported for each milling process (DD, dry degermination; TD, tempering degermination) as the percentage with respect to the raw material content (contamination of pre-cleaned whole grain = 100).