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Effect of milling procedures on mycotoxin distribution in wheat fractions: A review

Federica Cheli*, Luciano Pinotti, Luciana Rossi, Vittorio Dell'Orto

Department of Health, Animal Science and Food Safety, Università degli Studi di Milano, Via Trentacoste 2, 20134 Milano, Italy

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ABSTRACT

Cereals and cereal by-products constitute a major part of human and animal diet. It has been estimated that up to 25% of the world's crops may be contaminated with mycotoxins. The relevance of mycotoxins on human/animal health prompted the European Community to introduce maximum permissible limits in foods and feeds. Considering the levels indicated by the European legislation, results from literature indicate that sometimes the limits proposed for cereal-derived products may be not warranted by the limit for unprocessed cereals. Therefore, the understanding of how mycotoxin distribution and concentration change during the milling process is a worldwide topic of interest due to the high economic and health impact.

This paper reviews the most recent findings on the effects of wheat milling process on mycotoxin distribution in products and by-products. Published data confirm that milling can minimize mycotoxin concentration in fraction used for human consumption, but concentrate mycotoxins into fractions commonly used as animal feed. The concentration of mycotoxins in wheat by-products may be up to eight-fold compared to original grain. Other physical processes carried out before milling, such as sorting, cleaning, and debranning, may be very efficient to reduce the grain mycotoxin content before milling. Published data show a high variability in mycotoxin repartitioning and sometimes appear conflicting, but this may be mainly due to the type of mycotoxins, the level and extent of fungal contamination, and a failure to understand the complexity of the milling technology. A precise knowledge of such data is vital and may provide a sound technical basis to mill managers to conform to legislation requirements, support risk management and regulatory bodies in order to reduce human and animal exposure to mycotoxins, reduce the risk of severe adverse market and trade repercussions, and revise legislative limits.

1. Introduction

Cereals and cereal by-products constitute a major part of the daily diet of the human and animal populations. The end products of wheat processing are, other than semolina or flour, several by-products coming from the surface layers, characterised by higher micronutrient and bran contents, and mainly used as animal feeds. However, they may represent a source of compounds with unique physico-chemical, nutritional, and functional properties which may have a high value for human nutrition, too (Hemery, Rouau, Lullien-Pellerin, Barron, & Abecassis, 2007).

Among the most important risks associated to wheat product consumption are mycotoxins. Mycotoxins are fungal secondary metabolites that have a great impact on human and animal health (Hussein & Brasel, 2001). This prompted the European Community to establish appropriate maximum levels in foodstuffs and feedstuffs (Commission Directive 2003/100/EC; Commission Recommendation 2006/576/EC; Commission Regulation (EC) No 1881/2006; Commission Regulation (EC) No 1126/2007). Considering the levels indicated by the European legislation, results from literature indicate that sometimes the limits proposed for cereal-derived products may be not warranted by the limit for unprocessed cereals. Therefore, based on occurrence data, the European limits for mycotoxin in cereals could impact the availability of high fibre cereal products. The fate of mycotoxins during cereal processing, such as sorting, cleaning, milling and thermal processes, was studied by several Authors (Brera et al., 2006; Bullerman & Bianchini, 2007; Hazel & Patel, 2004; Kabak, 2009; Kushiro, 2008; Scudamore, 2008; Scudamore & Patel, 2008; Visconti & Pascale, 2010).

This paper reviews the most recent findings on the effects of the milling process on mycotoxin distribution in wheat milling

* Corresponding author. Tel.: +39 02 50315734; fax: +39 02 50315746.
E-mail address: federica.cheli@unimi.it (F. Cheli).

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fractions. A precise knowledge of such data is vital as they may provide a sound technical basis to mill manager and support risk management and regulatory bodies in order to reduce human and animal exposure to dangerous amounts of mycotoxins, and revise legislative limits.

2. Occurrence of mycotoxins in wheat: a safety, economic, and technological topic

FAO's latest estimates for world cereal production in the period 2011–2012 are approximately 2313 million tons (FAO, 2011). For the feed sector, cereals represent the main components of industrial feeds, which estimated production, worldwide, is more than 717 million tons (Best, 2011). It has been estimated that up to 25% of the world's crops grown for foods and feeds may be contaminated with mycotoxins (Hussein & Brasel, 2001). This means that, if the estimated world cereal production is about 2300 million tons (2011), there are potentially over 500 million tons of mycotoxin contaminated grain entering the feed and food supply chain.

The major mycotoxins occurring in wheat, at levels of potential concern for human and animal health, are *Fusarium* mycotoxins. Specific reviews reporting the worldwide occurrence of *Fusarium* toxins in foods and feeds are provided by several authors to whom the reader is directed (Binder, Tan, Chin, Handl, & Richard, 2007; Placinta, D'Mello, & Macdonald, 1999; Rodrigues & Naehrer, 2012; SCOOP TASK 3.2.10, 2003; Visconti & Pascale, 2010; Zinedine, Soriano, Moltó, & Mañes 2007). Some results regarding the occurrence of *Fusarium* toxins in wheat and wheat bran are reported in Table 1. Although mycotoxin contamination levels of wheat present a high variability between regions, years, weather conditions, as well as between varieties and sowing time, deoxynivalenol (DON) has been the most common mycotoxin contaminant of wheat and wheat-based products worldwide, followed by nivalenol (NIV), zearalenone (ZEA), T-2 and HT-2 toxins. Data on the occurrence of *Fusarium* mycotoxins in durum wheat are quite limited. Available data indicated that durum wheat was generally more contaminated than common wheat, but, with the exception of a few samples, no durum wheat sample was noncompliant to the maximum permitted level for DON and ZEA (Visconti & Pascale, 2010).

Globalisation of the trade in agricultural commodities and lack of legislative harmonization have contributed significantly to the discussion about the awareness of mycotoxins entering the food chain. Aside from health risks, important economic and trade implications arise from such contamination (Bryden, 2012; Dohlman, 2003; Wu, 2004). The economic costs and impact on the international trade, associated with mycotoxin contamination, are difficult to assess in a consistent and uniform way and impossible to determine accurately (Wu, 2004). The economic impact of mycotoxins, considering the seasonality of contamination for the different toxins, includes loss of crop production, disposal of contaminated foods and feeds, reduced livestock production, loss of human and animal life, increased human and animal health care costs, analytical and regulatory costs, and investment in research. Particularly, the evidence on human health outcomes is too slight to permit an accurate analysis. A few examples of estimated economic costs associated with mycotoxin contamination in different food products are reported in Table 2.

Food safety remains the primary concern with *Fusarium* toxins in wheat. However, the impact on wheat processing properties cannot be ignored, and must be considered when establishing mycotoxins limits. *Fusarium* damage has a detrimental effect on the quality and the processing performance of wheat. *Fusarium* damage may reduce wheat milling performance, affect flour yield and flour ash, with a strong negative effect on flour brightness, and baking performance (Lancova et al., 2008; Siuda, Grabowski, Lenc, Ralcewicz, & Spychaj-Fabisiak, 2010). Changes in enzyme activity after *Fusarium* infection may be responsible of the observed changes in wheat quality (Wang et al., 2005).

3. Fate of mycotoxins during wheat milling

3.1. Sorting and cleaning

Physical and mechanical processes, such as sorting and cleaning prior to milling, may reduce mycotoxin contamination in wheat, by removing kernels with extensive mould growth, broken kernels, fine materials, and dust (Bullerman & Bianchini, 2007; Hazel & Patel, 2004; Kushiro, 2008). This is the materials (screenings) in which most of the toxins accumulate (Pascale et al., 2011; Visconti, Haidukowski, Pascale, & Silvestri, 2004). The most recent findings regarding the effect of sorting and cleaning of wheat on mycotoxin repartitioning are reported in Table 3. Experiments were performed using various kind of wheat, naturally or artificially infected, with mycotoxin contamination from low (<100 μg/kg) to high levels (>10,000 μg/kg). Results indicate that the effect of pre-milling processes and the efficiency of mycotoxin removal are extremely variable. The concentration of mycotoxins in cleaned wheat, compared to that in unclean grains, has ranged from 7 to 63% for DON, from 7 to almost 100% for NIV, and from 7 to 40% for ZEA (Edwards et al., 2011; Lancova et al., 2008; Neuhof, Koch, Rasenko, & Nehls, 2008a). Pascale et al. (2011) reported a 62% and 53% reduction of T-2 and HT-2, respectively, in wheat grains after cleaning. Several factors may be involved in this response, such as the initial condition of the grains, the type and extent of the contamination, and the type of cleaning process. Various equipments for wheat cleaning are available. Selection can be made according to different properties of wheat kernels: shape, size, relative density, and air resistance. Therefore, according to the type and extent of contamination, not all the equipments are equally efficient. Grains heavily *Fusarium* infected become shrivelled and may have lower relative density than healthy grains. Therefore, these can be removed more efficiently by the use of gravity separators rather than by other technological approaches (Hazel & Patel, 2004). The potential practical application of different selection processes and spectrometric analysis, such as mid-infrared spectroscopy or fluorescence analysis, for the selection of *Fusarium*-infected kernels merits further studies (Delwiche, Pearson, & Brabec, 2005; Singh, Jayas, Paliwal, & White, 2009).

According to EU regulations, foodstuffs can be subjected to physical treatments to reduce mycotoxin contamination levels. Interestingly, differences in maximum limits are reported for unprocessed cereals placed on the market for first-stage processing and cereals for direct human consumption.

> First-stage processing shall mean any physical or thermal treatment, other than drying, of or on the grain. Cleaning, sorting and drying procedures are not considered to be 'first-stage processing' insofar no physical action is exerted on the grain kernel itself and the whole grain remains intact after cleaning and sorting. In integrated production and processing systems, the maximum level applies to the unprocessed cereals in case they are intended for first-stage processing (European Commission, 2006, p. 23).

This is an important point with practical implications in the real commercial situation. In the industrial practice, the cleaning equipment may be incorporated in the milling plant, and this makes extremely complex the collection of wheat samples at the right sampling point in order to apply the maximum limits reported in regulations. Failure to take this into account makes it 1)

Table 1
Results of surveys on worldwide mycotoxin occurrence in wheat and wheat bran.

Mycotoxins *(EU maximum levels, μg/kg[a])*	Continents/Countries		Food	% of positive samples[b] (total samples)	Content, μg/kg	References
DON *(UW: 1250; UDW: 1750; W and WB: 750)*	Europe	Europe and Mediterranean regions	W	62(254)	370–5510[c]	Binder et al., 2007
		Europe	W	–	4–20,500	Placinta et al., 1999
		Northern Europe	W/WB	55(71)		
		Central Europe	W/WB	55(436)	641–7341[c]	Rodrigues & Naehrer, 2012
		Southern Europe	W/WB	38(24)	514–49,000[c]	
		Austria	W	57(166)	716–3505[c]	
		Belgium	W	37(47)	Up to 6090	SCOOP TASK 3.2.10, 2003
		Finland	W	61(138)	Up to 504	
		France	W	99(57)	Up to 1026	
		France	SW	76(792)	Up to 2125	
		France	DW	73(97)	Up to 1900	
		Germany	W	80(26)	Up to 3600	
		The Netherland	W	59 (761)	Up to 764	
		The Netherland	DW	60 (41)	Up to 3280	
		Norway	W	57(993)	Up to 1700	
		Sweden	W	90(176)	Up to 2700	
	America	North America	W	–	100–10,500	Placinta et al., 1999
		South America	W/WB	76(25)	600–7000[c]	Rodrigues & Naehrer, 2012
			W	–	100–9250	Placinta et al., 1999
			W/WB	53(17)	906–2520[c]	Rodrigues & Naehrer, 2012
	Asia/Oceania	Asia	W	–	29–11,700	Placinta et al., 1999
		Asia and Oceania	W/WB	79(115)	Up to 2251	Rodrigues & Naehrer, 2012
		Oceania	W/WB	81(98)	98–18,991[c]	Binder et al., 2007
			W/WB	48(109)	719–49,307[c]	Rodrigues & Naehrer, 2012
ZEA *(UW: 100; W and WB: 750)*	Europe	Europe and Mediterranean regions	W	92(48)	62–921[c]	Binder et al., 2007
						Placinta et al., 1999
		Europe	W	–	1–8400	Zinedine et al., 2007
		Northern Europe	W	–	17–231	Rodrigues & Naehrer, 2012
			W/WB	15(71)	96–233[c]	
		Central Europe	W/WB	12(256)	65–336[c]	
		Southern Europe	W/WB	0(17)	–	SCOOP TASK 3.2.10, 2003
		Finland	W	4(138)	Up to 5.3	
		France	W	82(230)	Up to 174	
		Italy	W	0(53)	–	Zinedine et al., 2007
	America	North America	W	–	36–11,050	Rodrigues & Naehrer, 2012
		South America	W/WB	13(16)	274–513[c]	Placinta et al., 1999
			W	–	40–210	Rodrigues & Naehrer, 2012
			W/WB	47(32)	72,5–393[c]	Zinedine et al., 2007
			W	–	40–210	Rodrigues & Naehrer, 2012
	Asia/Oceania	Asia	W/WB	62(112)	Up to 6641	
		Oceania	W/WB	28(115)	179–23,278[c]	Binder et al., 2007
		Asia and Oceania	W/WB	26(98)	77–1498[c]	Zinedine et al., 2007
		Asia	W	–	0.18–1400	
		Oceania	W	–	40–430	
NIV	Europe	Europe	W	–	4–20,500	Placinta et al., 1999
		Austria	W	5(166)	Up to 270	SCOOP TASK 3.2.10, 2003
		Finland	W	3(134)	Up to 200	
		France	W	100(55)	Up to 285	
		France	SW	17(491)	Up to 230	
		France	DW	46(97)	Up to 300	
	America	South America	W	–	160–400	Placinta et al., 1999
	Asia	Asia	W	–	10–4400	
T-2	Europe	Europe and Mediterranean regions	W	22(83)	51–829[c]	Binder et al., 2007
		Finland	W	0(134)	–	SCOOP TASK 3.2.10, 2003
		France	W	100(52)	n.r.	
		France	SW	13(570)	50	
		France	DW	0(97)	–	
		Italy	W	100(54)	Up to 160	
	Asia/Oceania	Asia and Oceania	W/WB	1(86)	Up to 266	Binder et al., 2007
HT-2	Europe	Finland	W	1(134)	Up to 46.5	SCOOP TASK 3.2.10, 2003
		France	W	100(52)	n.r.	
		France	SW	12(256)	n.r.	
		France	DW	0(97)	–	
		Norway	W	1(284)	26	
Fumonisins	Europe	Europe and Mediterranean regions	W	100(1)	580	Binder et al., 2007
		Northern Europe	W/WB	0(1)	–	Rodrigues & Naehrer, 2012
		Central Europe	W/WB	33(9)	246–450[c]	
	America	Southern Europe	W/WB	30(10)	151–925[c]	

(*continued on next page*)

Table 1 (*continued*)

Mycotoxins *(EU maximum levels, μg/kg[a])*	Continents/Countries		Food	% of positive samples[b] (total samples)	Content, μg/kg	References
		North America	W/WB	0(7)	–	
	Asia/Oceania	South America	W/WB	5(40)	1407–1715[c]	
		Asia	W/WB	9(113)	Up to 874	
		Oceania	W/WB	12(109)	196–1196[c]	
		Asia and Oceania	W/WB	4(98)	243–646[c]	Binder et al., 2007
Aflatoxins *(W and WB - Aflatoxin B1: 2,0 Sum aflatoxins: 4,0)*	Europe	Europe and Mediterranean regions Northern Europe	W	0(11)	–	Binder et al., 2007
		Central Europe	W/WB	0(1)	–	Rodrigues & Naehrer, 2012
		Southern Europe	W/WB	31(13)	1.6–2[c]	
		North America	W/WB	43(14)	1.6–6.0[c]	
	America	South America	W/WB	20(15)	4.1–9.0[c]	
	Asia/Oceania	Asia	W/WB	3(40)	2.6–3.0[c]	
		Oceania	W/WB	7(116)	Up to 20	
		Asia and Oceania	W/WB	5(109)	2.0–7.0[c]	
			W/WB	0(97)	–	Binder et al., 2007
Ochratoxin *(OTA – UW: 5,0; W and WB: 3,0)*	Europe	Europe and Mediterranean regions	W	42(12)	2–7[c]	
		Northern Europe	W/WB	0(2)	–	Binder et al., 2007
		Central Europe	W/WB	23(22)	3.8–331[c]	Rodrigues & Naehrer, 2012
		Southern Europe	W/WB	8(13)	0.7–1.0[c]	
	America	North America	W/WB	50(2)	0.8–1[c]	
		South America	W/WB	45(11)	32.9–43[c]	
	Asia/Oceania	Asia	W/WB	25(107)	Up to 30	
		Oceania	W/WB	8(108)	1.6–4.0[c]	
		Asia and Oceania	W/WB	75(8)	4–23[c]	Binder et al., 2007

n.r.: not reported; W: wheat; UW: unprocessed wheat; SW: soft wheat; DW: durum wheat; UDW: unprocessed durum wheat; WB: wheat bran.
[a] Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs.
[b] Positive samples > LOD for each mycotoxins (for specific LOD see references).
[c] Median of positive samples – maximum level.

difficult to properly apply the legislative approach, and 2) possible to reject consignment meeting the legislative requirements. This underlines the need to collect data from in real milling situations to better support risk management and regulatory bodies in order to revise the current limits.

3.2. Debranning

Debranning of wheat, a mechanical process by which the outer layers of wheat grains are removed prior to the milling process, is used in industrial processing as it can enhance the milling

Table 2
Quantitative estimates of economic losses associated with mycotoxin contamination in different commodities: a few examples from Bryden (2012), Dohlman (2003), Wu (2004, 2007).

Commodities	Mycotoxin	Type of costs	Amount, annually (US$)
Agricultural commodities (USA)	Mycotoxins	Total losses	630 million to 2.5 billion
Corn, wheat, and peanuts (USA)	Aflatoxin, fumonisin, and DON	Crop losses Regulatory enforcement, testing, and quality control Livestock losses	932 million 466 million 6 million
Cereals, dried fruits and nuts (export from Africa to Europe)	Aflatoxins (standard set by EU legislation)	Export	670 million (reduced health risk by approximately 1.4 deaths per billion)
Peanut meal (export from India to Europe)	Aflatoxins (standard set by EU legislation)	Export	More than 30 million
Corn and peanuts (export from developing Asian countries)	Aflatoxins	Market loss Livestock losses Human health losses	200 million 200 million 500 million
Peanuts from Africa to Europe	Aflatoxins (standard set by EU legislation)	Export	670 million
Corn (export from USA, China, and Argentina to Europe)	Fumonisin (for a standard of 0.5 mg/kg adopted worldwide)	Export	300 million
Corn (used as animal feed)	Fumonisins	Market loss Animal life losses	0.917–19.3 million ("Normal" year of *Fusarium* ear rot) 126,000 ("Normal" year of *Fusarium* ear rot)
		Market loss Animal life losses	27.5–45.8 million ("Outbreak" year of *Fusarium* ear rot) 320,000 ("Outbreak" year of *Fusarium* ear rot)

Table 3
Effect of cleaning and sorting procedures on mycotoxin content in wheat: more recent findings.

Mycotoxin	Initial level, μg/kg	Cleaning procedure	Average reduction in cleaned wheat, %	References
DON	350–13,100	Rationel Kornservice Mod. M220V sifter (two sieves)	20	Visconti et al., 2004
DON	<1000 to >20,000	High-speed, optical sorter (ScanMaster II 2000 DE, Satake-USA, Houston, TX) (one-pass sorting)	49	Delwiche et al., 2005
DON NIV	92–2985 <10–81	Sieving, scouring and polishing	48 80–100	Lancova et al., 2008
DON NIV ZEA	160 1160 77	Manual according to visual attributes	63 44 40	Neuhof et al., 2008a
ZEA	74	Manual according to their visual attributes	33	Neuhof et al., 2008b
DON	61	Grain sieving, removal of foreign seeds, stones and dust	48	Scudamore & Patel, 2008
DON NIV ZEA	193–15,095 <20–384 <5–768	Preliminary and secondary cleaning	*average* 7	Edwards et al., 2011
T-2 HT-2	35–785 62–5169	Rationel Kornservice Model M220V sifter (two sieves)	62 53	Pascale et al., 2011

performance of wheat and the degree of refinement of flour and semolina (Dexter & Wood, 1996). The effects of laboratory and industrial debranning in reducing the level of mycotoxin content in debranned wheat are reported in Table 4. Results indicate that the effect of debranning and the efficiency of mycotoxin removal are extremely variable. The reduction of DON in debranned wheat ranged from 15 to 78% (Aureli & D'Egidio, 2007; Cheli et al., 2010; Rios, Pinson-Gadais, Abecassis, Zakhia-Rozis & Lullien-Pellerin, 2009; Sovrani et al., 2012). At a laboratory scale, the efficiency of debranning is irrespective of the initial level of mycotoxins in grains, depends on the length of the pearling process, and the percentage of grain tissue removal (Rios, Pinson-Gadais, et al., 2009). Moreover, some studies report that debranning before milling was more efficient than the classical milling process to reduce DON content in products from the starchy endosperm (Aureli & D'Egidio, 2007; Rios, Pinson-Gadais, et al., 2009). It was pointed out that pearling may avoid recontamination of semolina by the finest milling particles in which a higher DON concentrations was reported (Rios, Pinson-Gadais, et al., 2009; Rios, Zakhia-Rozis, et al., 2009). At the industrial level, more studies are needed to optimize debranning technical conditions in order to reduce at most mycotoxin contamination with less grain tissue removal.

3.3. Milling

As in cleaning and debranning, in the milling process there is no step that destroys mycotoxins; however mycotoxin contamination may be redistributed and concentrated in certain milling fractions. Mycotoxins tend to be concentrated in cereal milling fractions that are less likely to be used for food production, but mainly used as animal feeds. Regarding wheat, the knowledge of mycotoxin repartitioning in milling fractions is largely limited to DON (for a review, see Kushiro, 2008). The most recent findings regarding *Fusarium* mycotoxin repartitioning in wheat products and by-products are reported in Table 5. Experiments were performed using various kind of wheat, naturally or artificially infected, with different mycotoxin contamination and contamination levels, from a low (<35 μg/kg) to a high level (>24,100 μg/kg). Taking into account that the approach in each study was different, and that most of the studies were carried out on DON, all studies reported similar trends in mycotoxin distributions in the various milled-wheat fractions. Levels of mycotoxins are lower in fractions intended for human consumption (flour or semolina) and higher in fractions mainly intended for animal feeds (bran, flour shorts screenings and middlings). However, the variability of the reported distribution factors is very high. The concentration of mycotoxins in white flour or semolina, compared to that in the wheat grain, has ranged from 11% to 89%, for T-2/HT2 and DON respectively (Pascale et al., 2011; Thammawong et al., 2011), but more typically ranges from 50 to 70%. The retention levels in semolina were shown to depend on the variety of wheat, the penetration degree of *Fusarium* moulds, and a transfer of mycotoxins to the inner parts of the kernel (Pinson-Gadais et al., 2007). Other Authors reported a low capacity of DON producing fungi to penetrate to the inner part of the grain

Table 4
Effect of debranning on mycotoxin content in wheat.

Mycotoxin	Initial level, μg/kg	Debranning procedure	Average reduction in debranned wheat, %	References
DON	684 250 135	Laboratory discontinuous debranner SB-SA (Colombini)	78 74 [a]	Aureli & D'Egidio, 2007
DON	382 4203	Abrasive laboratory mill (Satake Model TM-05C) 35% grain tissue removal	69 64	Rios, Pinson-Gadais, et al., 2009
DON	68.58	PeriTec, Satake Corporation, Jp	15	Cheli et al., 2010
DON	n.r.	Abrasive laboratory mill (Satake model TM-05C) 10% grain weight removal	64	Sovrani et al., 2012

[a] DON level in debranned wheat below detection limit.

Table 5
Repartitioning of mycotoxins in wheat milling fractions: more recent findings.

Mycotoxin	Wheat μg/kg	Milling procedure	Flour	Semolina	By-products		References
			Distribution factor[a], average %		Type	Distribution factor[a], average %	
DON	350–13,100	Bühler laboratory mill (MLU-202)		37	Bran Screenings Fine middlings	159 414 64	Visconti et al., 2004
DON	684 250 135	Bühler laboratory mill (MLU-202)		60 75 25	Middling	145 101 62	Aureli & D'Egidio, 2007
DON	684 250 135	Laboratory discontinuous debranner SB-SA + Bühler laboratory		35 45 35	Middling	87 78 60	
DON ZEA DON ZEA	n.r. n.r. n.r. n.r.	Stone mill Roller mill 	170 μg/kg[b] 6 μg/kg[b] 360 μg/kg[b] 13 μg/kg[b]				Palpacelli et al., 2007
DON	750 8000	Semi-industrial semolina mill		67 44			Pinson-Gadais et al., 2007
DON	92–2985	Bühler laboratory mill (MLU-202)	45		Bran	105	Lancova et al., 2008
DON	19–481	Industrial milling	50[c]		Bran	340[c]	Scudamore, 2008
DON	24,100	Experimental milling, Chopin CD1 mill		87	Bran	153	Herrera et al., 2009
DON	382 4203	Semi-industrial semolina mill		58 70	Total bran and shorts Total bran and shorts	238 216	Rios, Zakhia-Rozis, et al., 2009
DON	68.58	Industrial conventional milling		55	Shorts (middling) Flour shorts	152 118	Cheli et al., 2010
DON	68.58	Industrial debranning milling		60	Shorts (middling) Flour shorts	308 178	
DON NIV	896 722	Bühler laboratory mill (MLU-202)	89 78		Bran Shorts Bran Shorts	81 174 78 140	Thammawong et al., 2010
DON ZEA	87 11	Industrial milling	70[c] 44[c]		Bran Bran	282[c] 360[c]	Edwards et al., 2011
DON	82–1700	Bühler laboratory mill (MLU-202)	50		Bran	158	Kostelanska et al., 2011
NIV	300	Bühler laboratory mill (MLU-202)	47		Bran Shorts	180 280	Thammawong et al., 2011
T-2	35–785	Bühler laboratory mill (MLU-202)		11	Bran Screenings Fine middlings	171 800 23	Pascale et al., 2011
HT-2	62–5169			11	Bran Screenings Fine middlings	370 540 21	

n.r.: not reported.
[a] Distribution factor: percentage of overall reduction or increase in DON content for each milling fraction in comparison with initial level in unprocessed wheat.
[b] Mean mycotoxin content in the stone-mill and roller-mill flours.
[c] Distribution factor: percentage of overall reduction or increase in DON content for each milling fraction in comparison with initial level in cleaned wheat.

(Rios, Zakhia-Rozis, et al., 2009). DON and ergosterol, an important metabolite used as an indicator of fungal biomass in grains, were found to be similarly partitioned by milling and positively correlated, suggesting that the fungal infection was greater at or near the surface of the kernels and that the mycotoxin was produced at the site of fungal growth rather than transported from the kernel surface to the interior (Barajas-Aceves, Hassan, Tinoco, & Vazquez-Duhalt, 2002). The concentration of mycotoxins in some wheat milling fractions, compared to that in the wheat grain, may be up to 800%, but more typically ranges from 150 to 340% (Table 5). This is an important topic because of the main use of these by-products in feed production. Factors that cause this variability have not been completely determined. The high mycotoxin repartitioning in wheat by-products may indicate a concentration of toxins in the outer part of the kernel (Lancova et al., 2008). Peripheral tissue, such as the pericarp and testa, are the parts of the grain first colonized by fungi and often contaminated by microorganisms (Brera et al., 2006; Hemery et al., 2007). However, mycotoxin contamination of milling by-products may not simply be due to the presence of peripheral grain tissues. When ash, phytic acid, and crude fibre were used as markers to monitor the presence of external tissues, even if a highest concentration of DON was found

in fractions originating from the grain outer layers, a lack of correlation was found with ash and phytic acid, and a low positive correlation with fibre (Cheli et al., 2010; Greffeuille, Abecassis, Bar L'Helgouach, & Lullien-Pellerin, 2005; Rios, Pinson-Gadais, et al., 2009; Rios, Zakhia-Rozis, et al., 2009). Despite a similar behaviour in mycotoxin repartitioning in wheat by-products, significant difference between distribution factors were observed. After inoculation by toxigenic *Fusarium* strains, semolina was shown to allow high yields of trichothecenes, while bran was demonstrated to contain biochemical inhibitors able to significantly reduce trichothecene production (Pinson-Gadais et al., 2007). Bran accumulates more HT-2 than T-2, while fine middlings and semolina more T-2 than HT-2 (Pascale et al., 2011). The presence of carboxylesterase enzymes, with specific activity towards T-2 to produce HT-2 in cereals has recently been shown, and the potential role of this hydrolytic activity in the detoxification plant response to T-2 cannot be excluded. Rios, Zakhia-Rozis, et al. (2009) reported that there is a different distribution of DON in the outcoming milling fractions according to the fraction size, with finest particles being more contaminated. The repartitioning of these finest particles during the milling processing steps may be another factor affecting the variability of mycotoxin repartitioning in wheat by-products. This is an important topic so much so that it was considered by the legislator. Different maximum levels were set for *Fusarium* toxins in maize and maize products milling fractions, not used for direct human consumption, according to the particle size (European Commission, 2007). Another factor affecting the degree to which *Fusarium* toxins in cereals are removed/concentrated by milling is definitely the milling method. Laboratory studies, pilot-scale and industrial milling technologies may not be equivalent (Aureli & D'Egidio, 2007; Cheli et al., 2010; Lancova et al., 2008; Palpacelli, Beco, & Ciani, 2007).

4. Conclusions and future perspective/trends/needs

Industrial milling technology is a very complex process and presents several key processing steps that differently influence mycotoxin repartitioning in wheat milling fractions. Published data confirm that milling reduces mycotoxin concentration in fractions used for human consumption, but concentrates mycotoxins into fractions commonly used as animal feed. However, these fractions may represent promising novel food ingredients with a high value for human nutrition, too. Physical processes carried out before milling (such as sorting, cleaning, debranning) are interesting and efficient methods to reduce the grain mycotoxin content before milling. These processes may be even more efficient than conventional milling. A high variability in mycotoxin repartitioning was reported and sometimes inconsistent results were reported. This may be mainly due to the type of mycotoxins, the level and extent of fungal contamination, and an omission of the description of the complexity of milling technology. Therefore, factors that influence this distribution, such as technical milling procedures, biology of mycotoxins and fungi, deserve further studies. Regarding these topics, there is a need of more studies carried out at industrial scale. Studies on pilot or laboratory scale may provide good prediction models, but do not guarantee that this exactly occurs in real commercial situation, where the acceptance of wheat meeting the legislative requirements could be challenged later in the food chain when the expected reduction in mycotoxin concentration is not achieved. Moreover, an important topic, when studying repartitioning of mycotoxins during milling at industrial scale, is to consider the sampling procedures of cereals in order to respect the final purposes, i.e. fixed maximum tolerable levels or other operational targets for food/feed industry. The heterogeneous distribution of mycotoxin contamination has great implications for a precise evaluation of the actual mycotoxin level in whole grain kernels and milling by-products (Whitaker, 2004). Cereals bulk moisture usually facilitates the development of localized clumps particularly rich in moulded kernels. These small percentages of extremely contaminated portions ("hot spots") are randomly distributed in a lot (average value usually registered about 0.1%) (Johansson, Whitaker, Giesbrecht, Hagler, & Young, 2000). This condition can lead to an underestimation of the real level of mycotoxin if a too small sample size without contaminated particles is analysed or, instead, to an overestimation of the true level in the case of a too small sample size featuring or more contaminated particles. Thus, major sources of error in the analysis of mycotoxins arise from inadequate sampling (Cheli, Campagnoli, Pinotti, Fusi, & Dell'Orto, 2009). Therefore, the plan of an effective sampling procedure for cereal mycotoxin detection or quantification represents a complex challenge for operators. The methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs and feed are laid down in Commission Regulation (EC) No 401/2006 and Commission Regulation (EC) No 152/2009, respectively.

The knowledge of mycotoxin repartitioning in wheat milling fractions is largely limited to DON, using different approaches (artificially *vs.* naturally contaminated wheat; wide range of mycotoxin contamination levels; laboratory, semi-industrial, and industrial milling), but there is still a lack of data for other mycotoxins. The co-occurrence of several mycotoxins, with specific chemical traits and modes of action, is a serious problem because of potential additive and/or synergistic effects. Future attention should be paid not only to the mycotoxins believed to be the most likely to occur, but also to less common toxins. The impact of mycotoxins entering the food chain could increase in the next future. Most predictions indicate that the climate change scenarios, with a global warming, could affect agriculture and increase the threat from fungal invasion of crops (Wu et al., 2011).

Further researches regarding mycotoxin repartitioning in industrial milling conditions will allow data collection to create an appropriate and reliable database that may provide a sound technical basis to mill managers to conform to legislation requirements, and define specific mill operative protocols according to the level of mycotoxin contamination and requirements of ingredients with specific properties. Moreover, this may represent a systematic basis for setting up a system for transmission and sharing of information to support risk management in wheat supply chain. This is essential for regulatory bodies in order to better assess the dietary intake, reduce human and animal exposure to mycotoxins, reduce the risk of severe adverse market and trade repercussions, and revise legislative limits.

References

Aureli, G., & D'Egidio, M. G. (2007). Efficacy of debranning on lowering of deoxynivalenol (DON) level in manufacturing processes of durum wheat. *Tecnica Molitoria, 58*, 729–733.

Barajas-Aceves, M., Hassan, M., Tinoco, R., & Vazquez-Duhalt, R. (2002). Effect of pollutants on the ergosterol content as indicator of fungal biomass. *Journal of Microbiological Methods, 50*, 227–236.

Best, P. (2011). World Feed Panorama: expensive grain slows industry expansion. *Feed International, 32*, 10–12.

Binder, E. M., Tan, L. M., Chin, L. J., Handl, J., & Richard, J. (2007). Worldwide occurrence of mycotoxins in commodities, feeds and feed ingredients. *Animal Feed Science and Technology, 137*, 265–282.

Brera, C., Catano, C., De Santis, B., Debegnach, F., De Giacomo, M., Pannunzi, E., et al. (2006). Effect of industrial processing on the distribution of aflatoxins and zearalenone in corn-milling fractions. *Journal of Agricultural and Food Chemistry, 54*, 5014–5019.

Bryden, W. L. (2012). Mycotoxin contamination of the feed supply chain: implications for animal productivity and feed security. *Animal Feed Science and Technology, 173*, 134–158.

Bullerman, L. B., & Bianchini, A. (2007). Stability of mycotoxins during food processing. *International Journal of Food Microbiology, 119*, 140–146.

Cheli, F., Campagnoli, A., Pinotti, L., Fusi, E., & Dell'Orto, V. (2009). Review article – sampling feed for mycotoxins: acquiring knowledge from food. *Italian Journal of Animal Science, 8*, 5–22.

Cheli, F., Campagnoli, A., Ventura, V., Brera, C., Berdini, C., Palmaccio, E., et al. (2010). Effects of industrial processing on the distributions of deoxynivalenol, cadmium and lead in durum wheat milling fractions. *LWT – Food Science and Technology, 43*, 1050–1057.

Commission Directive 2003/100/EC of 31 October 2003 amending Annex I to Directive 2002/32/EC of the European Parliament and of the Council on undesirable substances in animal feed. (1/11/2003). Commission Directive 2003/100/EC. *Official Journal of the European Union L 285*, 33–37.

Commission Recommendation 2006/576/EC of 17 August 2006 on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding. *Official Journal of the European Union L 229*, 7–9.

Commission Regulation (EC) No 1126/2007 of 28 September 2007 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards *Fusarium* toxins in maize and maize products. *Official Journal of the European Union L 255*, 14–17.

Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed. (26.2.2009). *Official Journal of the European Union, L 54*, 1–130.

Commission Regulation EC No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. *Official Journal of the European Union L 364*, 5–24.

Commission Regulation (EC) No 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs. (9.3.2006). *Official Journal of the European Union, L 70*, 12–34.

Delwiche, S. R., Pearson, T. C., & Brabec, D. L. (2005). High-speed optical sorting of soft wheat for reduction of deoxynivalenol. *Plant Disease, 89*, 1214–1219.

Dexter, J. E., & Wood, P. J. (1996). Recent applications of debranning of wheat before milling. *Trends in Food Science & Technology, 7*, 35–41.

Dohlman, E. (2003). Mycotoxin hazards and regulations impacts on food and animal feed crop trade. In J. C. Buzby (Ed.), *International trade and food safety economic theory and case studies* (pp. 97–108). USDA, Agricultural Economic Report, Number 828. Retrieved from United State Department of Agriculture website: http://www.ers.usda.gov/publications/aer-agricultural-economic-report/aer828.aspx.

Edwards, S. G., Dickin, E. T., MacDonald, S., Buttler, D., Hazel, C. M., Patel, S., et al. (2011). Distribution of *Fusarium* mycotoxins in UK wheat mill fractions. *Food Additives and Contaminants Part A – Chemistry Analysis Control Exposure & Risk Assessment, 28*, 1694–1704.

FAO (Food and Agriculture Organization of the United Nations). (July 2011). Cereal supply and demand brief. In *FAO cereal supply and demand situation*Available at http://www.fao.org/worldfoodsituation/wfs-home/csdb/en/.

Greffeuille, V., Abecassis, J., Bar L'Helgouach, C., & Lullien-Pellerin, V. (2005). Differences in the aleurone layer fate between hard and soft common wheats at grain milling. *Cereal Chemistry, 82*, 138–143.

Hazel, C. M., & Patel, S. (2004). Influence of processing on trichothecene levels. *Toxicology Letters, 153*, 51–59.

Hemery, Y., Rouau, X., Lullien-Pellerin, V., Barron, C., & Abecassis, J. (2007). Dry processes to develop wheat fractions and products with enhanced nutritional quality. *Journal of Cereal Science, 46*, 327–347.

Herrera, M., Juan, T., Estopanan, G., & Arino, A. (2009). Comparison of deoxynivalenol, ochratoxin A and aflatoxin B1 levels in conventional and organic durum semolina and the effect of milling. *Journal of Food and Nutrition Research, 48*, 92–99.

Hussein, H. S., & Brasel, J. M. (2001). Toxicity, metabolism, and impact of mycotoxins on humans and animals. *Toxicology, 167*, 101–134.

Johansson, A. S., Whitaker, T. B., Giesbrecht, F. G., Hagler, W. M., & Young, J. H. (2000). Testing shelled corn for aflatoxin, part III: evaluating the performance of aflatoxins sampling plans. *Journal of the Association of Official Analytical Chemists International, 83*, 1279–1284.

Kabak, B. (2009). The fate of mycotoxins during thermal food processing. *Journal of the Science of Food and Agriculture, 89*, 549–554.

Kostelanska, M., Dzuman, Z., Malachova, A., Capouchova, I., Prokinova, E., Skerikova, A., et al. (2011). Effects of milling and baking technologies on levels of deoxynivalenol and its masked form deoxynivalenol-3-glucoside. *Journal of Agricultural and Food Chemistry, 59*, 9303–9312.

Kushiro, M. (2008). Effects of milling and cooking processes on the deoxynivalenol content in wheat. *International Journal of Molecular Sciences, 9*, 2127–2145.

Lancova, K., Hajslova, J., Kostelanska, M., Kohoutkova, J., Nedelnik, J., Moravcova, H., et al. (2008). Fate of trichothecene mycotoxins during the processing: milling and baking. *Food Additives and Contaminants, 25*, 650–659.

Neuhof, T., Koch, M., Rasenko, T., & Nehls, I. (2008a). Occurrence of zearalenone in wheat kernels infected with *Fusarium culmorum*. *World Mycotoxin Journal, 1*, 429–435.

Neuhof, T., Koch, M., Rasenko, T., & Nehls, I. (2008b). Distribution of trichothecenes, zearalenone, and ergosterol in a fractionated wheat harvest lot. *Journal of Agricultural and Food Chemistry, 56*, 7566–7571.

Palpacelli, V., Beco, L., & Ciani, M. (2007). Vomitoxin and zearalenone content of soft wheat flour milled by different methods. *Journal of Food Protection, 70*, 509–513.

Pascale, M., Haidukowski, M., Lattanzio, V. M. T., Silvestri, M., Ranieri, R., & Visconti, A. (2011). Distribution of T-2 and HT-2 toxins in milling fractions of durum wheat. *Journal of Food Protection, 74*, 1700–1707.

Pinson-Gadais, L., Barreau, C., Chaurand, M., Gregoire, S., Monmarson, M., & Richard-Forget, F. (2007). Distribution of toxigenic *Fusarium* spp. and mycotoxin production in milling fractions of durum wheat. *Food Additives and Contaminants, 24*, 53–62.

Placinta, C. M., D'Mello, J. P. F., & Macdonald, A. M. C. (1999). A review of worldwide contamination of cereal grains and animal feed with *Fusarium* mycotoxins. *Animal Feed Science and Technology, 78*, 21–37.

Rios, G., Pinson-Gadais, L., Abecassis, J., Zakhia-Rozis, N., & Lullien-Pellerin, V. (2009). Assessment of dehulling efficiency to reduce deoxynivalenol and *Fusarium* level in durum wheat grains. *Journal of Cereal Science, 49*, 387–392.

Rios, G., Zakhia-Rozis, N., Chaurand, M., Richard-Forget, F., Samson, M. F., Abecassis, J., et al. (2009). Impact of durum wheat milling on deoxynivalenol distribution in the outcoming fractions. *Food Additives and Contaminants Part A – Chemistry Analysis Control Exposure & Risk Assessment, 26*, 487–495.

Rodrigues, I., & Naehrer, K. (2012). A three-year survey on the worldwide occurrence of mycotoxins in feedstuffs and feed. *Toxins, 4*, 663–675.

Scudamore, K. A. (2008). Fate of *Fusarium* mycotoxins in the cereal industry: recent UK studies. *World Mycotoxin Journal, 1*, 315–323.

Scudamore, K. A., & Patel, S. (2008). The fate of deoxynivalenol and fumonisins in wheat and maize during commercial breakfast cereal production. *World Mycotoxin Journal, 1*, 437–448.

SCOOP Task 3.2.10-Collection of occurrence data of *Fusarium* toxins in food and assessment of dietary intake by the population of EU member states. (April 2003). http://ec.europa.eu/food/fs/scoop/task3210.pdf.

Singh, C. B., Jayas, D. S., Paliwal, J., & White, N. D. G. (2009). Detection of sprouted and midge-damaged wheat kernels using near-infrared hyperspectral imaging. *Cereal Chemistry, 86*, 256–260.

Siuda, R., Grabowski, A., Lenc, L., Ralcewicz, M., & Spychaj-Fabisiak, E. (2010). Influence of the degree of fusariosis on technological traits of wheat grain. *International Journal of Food Science and Technology, 45*, 2596–2604.

Sovrani, V., Blandino, M., Scarpino, V., Reyneri, A., Coïsson, J. D., Travaglia, F., et al. (2012). Bioactive compound content, antioxidant activity, deoxynivalenol and heavy metal contamination of pearled wheat fractions. *Food Chemistry, 135*, 39–46.

Thammawong, M., Okabe, M., Kawasaki, T., Nakagawa, H., Nagashima, H., Okadome, H., et al. (2010). Distribution of deoxynivalenol and nivalenol in milling fractions from *Fusarium*-infected Japanese wheat cultivars. *Journal of Food Protection, 73*, 1817–1823.

Thammawong, M., Okadome, H., Shiina, T., Nakagawa, H., Nagashima, H., Nakajima, T., et al. (2011). Distinct distribution of deoxynivalenol, nivalenol, and ergosterol in *Fusarium*-infected Japanese soft red winter wheat milling fractions. *Mycopathologia, 172*, 323–330.

Visconti, A., Haidukowski, E. M., Pascale, M., & Silvestri, M. (2004). Reduction of deoxynivalenol during durum wheat processing and spaghetti cooking. *Toxicology Letters, 153*, 181–189.

Visconti, A., & Pascale, M. (2010). An overview on *Fusarium* mycotoxins in the durum wheat pasta production chain. *Cereal Chemistry, 87*, 21–27.

Wang, J. H., Wieser, H., Pawelzik, E., Weinert, J., Keutgen, A. J., & Wolf, G. A. (2005). Impact of the fungal protease produced by *Fusarium culmorum* on the protein quality and breadmaking properties of winter wheat. *European Food Research and Technology, 220*, 552–559.

Whitaker, T. B. (2004). Sampling for mycotoxins. In N. Magan, & M. Olsen (Eds.), *Mycotoxins in food: Detection and control* (pp. 69–81). Cambridge, UK: Woodhead Publishing Ltd.

Wu, F. (2004). Mycotoxin risk assessment for the purpose of setting international regulatory standards. *Environmental Science & Technology, 38*, 4049–4055.

Wu, F. (2007). Measuring the economic impacts of *Fusarium* toxins in animal feeds. *Animal Feed Science and Technology, 137*, 363–374.

Wu, F., Bhatnagar, D., Bui-Klimke, T., Cardone, I., Hellmich, R., Munkvold, G., et al. (2011). Climate change impacts on mycotoxin risks in US maize. *World Mycotoxin Journal, 4*, 79–93.

Zinedine, A., Soriano, J. M., Moltó, J. C., & Mañes, J. (2007). Review on the toxicity, occurrence, metabolism, detoxification, regulations and intake of zearalenone: an oestrogenic mycotoxin. *Food and Chemical Toxicology, 45*, 1–18.