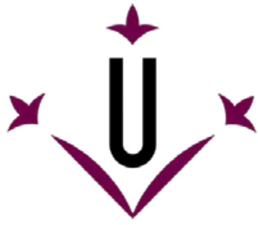

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6. Study I. Determination of aflatoxins, deoxynivalenol, ochratoxin A and zearalenone in wheat and oat based bran supplements sold in Spain's markets.

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6. Determination of aflatoxins, deoxynivalenol, ochratoxin A and zearalenone in wheat and oat based bran supplements sold in the Spanish market.

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6.1. Abstract

The aflatoxins (Afs), deoxynivalenol (DON), ochratoxin A (OTA) and zearalenone (ZEN) are mycotoxins produced by fungal species which can contaminate, alone or simultaneously, cereal-based raw materials. Usually, the higher mycotoxins concentrations in cereals are found in the external layers of the grain (bran). Nowadays bran is increasingly consumed for its high fiber concentration. The objectives of this study were determining the concentration of these mycotoxins in bran samples intended for direct human consumption and to study the influence of some characteristics of the samples that may affect the mycotoxins content, there aren't studies about fiber for direct human consumption. 67 bran samples from shops and supermarkets from

two different Spanish cities were analyzed, being 37 samples of wheat bran and the remaining of oat bran. The results showed a major presence of DON in the analyzed samples, with levels above the EU legislation in some samples. Presence of DON was more frequent in wheat samples, compared to oats ones ($p<0.05$). Extruded or toasted samples, subjected to a heat treatment during processing, presented a significantly lower concentration of OTA, and differences between the organically and conventionally produced samples were also detected in OTA, which showed higher levels in the organic samples. Co-occurrence was frequently found between the Fusarium mycotoxins (ZEN and DON). Due to the high levels of DON in the analyzed samples, a calculation of DON intake has been made and it has been demonstrated that bran can account for an important percentage of DON exposure in the total diet.

Keywords: aflatoxins, deoxynivalenol, ochratoxin A, zearalenone, dietary fiber.

6.2. Introduction

To date, over 300 mycotoxins have been identified, but the most important groups in foods are produced by these three genera: aflatoxins (AFs), produced by some Aspergillus species, ochratoxin A (OTA) produced by both Aspergillus and Penicillium, and Fusarium toxins, mainly trichothecenes (type A: HT-2 and T-2 toxin, and type B: deoxynivalenol (DON)), zearalenone (ZEN) and fumonisins B1 and B2. Moreover, Fusarium mycotoxins can be found alone or simultaneously, as well as co-occurring with other mycotoxins such as AFs, in cereals and in cereal-based foods (Jestoi, 2008). The co-occurrence is a usual situation in cereals, especially in mycotoxins potentially produced by the same fungal specie.

AFs are the most potent mutagenic and carcinogenic natural substances known. There are 6 types of AFs that frequently contaminate foods: B1, B2, G1, G2, M1 and M2. although the latter two are only found in the milk and derivatives. Aflatoxin B1 (AFB1) is consistently carcinogenic and genotoxic in vitro and in vivo (EFSA, 2007), and therefore it was classified in the group 1 by the International Agency for Research on Cancer (IARC, 2002). OTA is a nephrotoxic mycotoxin which possesses carcinogenic, teratogenic, immunotoxic and possibly neurotoxic properties (SCF, 1998). This mycotoxin has been classified as a possible human carcinogen, in the group 2B, by IARC (IARC, 2002). DON is one of the most regular contaminants in cereals (Jelinek et al., 1989), also known as vomitoxin. Although no indication of carcinogenic or mutagenic effects has been reported, DON has been linked with human gastroenteritis (Pestka, 2010a; Pestka 2010b). At the molecular level, DON disrupts normal cell function by inhibiting protein synthesis, affecting cell signalling, differentiation, and proliferation. An acute and high dose of DON can induce vomiting, whereas chronic dietary exposure to DON causes reduced feed intake, decreased nutritional efficiency, reduced weight gain, and immune dysregulation. It is the predominant mycotoxin with highest levels detected in the bran fraction (Edwards et al., 2011). ZEN is a non-steroidal estrogenic toxin, which the International Agency for Research on

Cancer (IARC) has categorized as a class 3 carcinogen (IARC, 1993). ZEN produces estrogenic effects in humans and animals leading to hyperestrogenism. ZEN can act as an estrogen analog and in humans has been recently considered as a triggering factor for central precocious puberty at least in prepubertal girls (Massart and Saggese, 2010).

Due to their toxicological effects, the European Commission has set maximum permitted levels in processed cereal products for direct human consumption: 2 μg/kg for AFB1 and 4 μg/kg for the sum of AFB1, AFG1, AFB2 and AFG2; 3 μg/kg for OTA, 750 μg/kg for DON and 75 μg/kg for ZEN (European Commission, 2006a, 2010).

Dietary fiber refers to plant cell wall components, mainly polysaccharides and is not digestible by human or other mammalian digestive enzymes. The benefits of dietary fiber intake are numerous, ranging from improved large bowel function to slowed digestion and absorption of carbohydrate and fat and reduced risk for certain diseases. Based on the effects on bowel function, the EFSA Panel considered fiber intake of 25 g/day to be adequate for normal laxation in adults (EFSA, 2010). Despite of the widely reported benefits of fiber intake, a study showed that Spanish population only eats 16.5 g/day (Ruiz-Roso and Pérez, 2010). Because of the low levels of fiber intake, some fiber supplements can be found in the market. Most of the fiber supplements are based on cereal bran (the bran is the external layer of the grain and it is obtained as the result of abrasion), because it is cheap and contain a high level of fiber. However, cereal bran is the part of the grain with the highest concentration of mycotoxins (Thammawong et al., 2010). Wheat bran is the most common source of dietary fiber. In recent years, due to the wide spreading of several slimming diets, like Dukan diet, the consumption of bran has increased. For example, a rule of Dukan diet is that high quantities of oat bran must be eaten everyday (Hansel et al., 2011), usually up to 3 spoonfuls daily, which in some cases could represent more than 60 grams per day.

There are few researches on the presence of mycotoxins in oat and wheat bran and, to our knowledge, none dealt with dietary bran for human consumption. This is the first research with fiber intake. The objectives of this paper were, in one hand, to determine the AFs, OTA, DON and ZEN levels of oat and wheat bran commercially available in the Spanish market and, on the other hand, to assess the safety of this type of product for usual consumers.

6.3. Materials and methods

6.3.1. Cereal bran samples

Bran samples were purchased in 2012 from different shops from two different Spanish cities, Lleida and Valencia. The total number of samples was 67. They were packaged in plastic bags and pack weight ranged from 0.2 to 1 kg. There were 37 samples of wheat bran and 30 samples of oat bran. The different samples were taken from hypermarkets or supermarkets and

health food stores. The samples were transported and stored under cool conditions until analysis (mean aw of the samples was 0.42). Full sample details including the type of cereal, the processing treatments, and the type of production, if available, were recorded for later statistical analysis.

6.3.2. Chemicals and reagents

Mycotoxin standards, including ZEN , DON, OTA, AFB1, AFB2, AFG1 and AFG2 were supplied by Sigma (Sigma-Aldrich, Alcobendas, Spain). Acetonitrile, methanol and ethanol were purchased from J.T. Baker (Deventer, The Netherlands). Benzene and n-hexane were purchased from Merck (Damstadt, Germany). All solvents were LC grade. Filter papers (Whatman number 1) and glass microfiber filters (Whatman GF/A) where purchased from Whatman (Maidstone, UK). Immunoaffinity columns (IAC) for AFs (Easi-extract® Aflatoxin), DON (DONPREP®) and ZEN (Easi-extract Zearalenone®) extracts clean-up were purchased from R-Biopharm (Rhône LTD Glasgow, UK). Pure water was obtained from a Milli-Q apparatus (Millipore, Billerica, MA, USA) and was used when water was required. Phosphate buffer saline (PBS) was prepared with potassium chloride (0.2 g) (Panreac, Castellar del Vallès, Spain), potassium dihydrogen phosphate (0.2 g) (Sigma), disodium phosphate anhydrous (1.16 g) (Panreac) and sodium chloride (8.0 g) (J.T Baker) in 1 L of pure water; the pH was brought to 7.4.

6.3.3. Preparation of standard solutions

The standards of ZEN, OTA, AFB1, AFB2, AFG1 and AFG2 were dissolved in methanol at a concentration of 5.0 mg/mL and stored at 4 ºC in a sealed vial until use. The concentration in the stock solutions was checked by UV spectroscopy according to AOAC Official methods of analysis chapter 49 (Horwitz, 2006). Working standard solutions (2.0, 1.0, 0.5, 0.1, 0.05, 0.01 and 0.005 µg/mL) were prepared by appropriate dilution of known volumes of the stock solution with mobile phase and used to obtain calibration curves in the appropriated chromatographic system. The standard of DON was dissolved in ethanol at a concentration of 10.0 mg/mL and stored at 4 ºC in a sealed vial until use. Working standards (10.0, 5.0, 1.0, 0.5, 0.1 and 0.05 µg/mL) were prepared by appropriate dilution of known volumes of the stock solution with mobile phase and used to obtain calibration curves in the appropriate chromatographic system.

6.2.4. Analytical methods

6.3.4.1. AFs and OTA

Five g of ground bran sample was mixed with 30 mL of extractant solution (60% acetonitrile, 40% water) for 10 min and filtered with filter Whatman number 1. 2 mL of filtered solution was diluted with 18 mL of PBS solution and drained through the IAC corresponding column. After this, the columns were washed with 20 mL of PBS and AFs and OTA were eluted by applying 1 mL of methanol grade HPLC and 1 mL of milli-Q water, consecutively. The equipment used for the HPLC simultaneous detection of AFs and OTA was a separation Module Alliance 2695 Waters®, an analytical column Water Spherisorb® 5mm ODS2, 4.6 x 250 mm and a Multi λ Fluorescence Detector Waters 2475®. Excitation and emission wavelengths were set, respectively, at 365 nm and 455 nm for AFs (0 to 16 minutes), and 333 nm and 463 nm for OTA (16 to 25 minutes). Derivatization of aflatoxins was obtained using a post-column photochemical derivatization device (UVETM Derivatizer LC Tech, Germany). Mobile phase consisted of methanol, acetonitrile and acetic acid 0.1 %, using the following proportions: 27 % of methanol, 14 % of acetonitrile and 59 % of acetic acid 0.1 %, until minute 16, and then 50 % of methanol and 50 % of acetonitrile until the end of the run. The mobile phase flow rate was 0.8 mL/min. The injection volume was 100 µL.

6.3.4.2. DON

Five g of ground bran sample was mixed with 40 mL of distilled water for 10 min. Then the sample was centrifuged for 8 min at 1780 *g*. Supernatant was filtered through a glass microfiber filter. 5 mL of filtered sample was drained through the IAC column and washed with 10 mL of distilled water. DON was eluted by applying 1.5 mL of methanol grade HPLC and 1.5 mL of milli-Q water, consecutively. The purified samples were dried under nitrogen stream. Each dried sample was resuspended with the mobile phase solution (water:acetonitrile:methanol, 92:4:4). DON was determined by HPLC coupled with a UV/Visible dual λ absorbance Detector Waters 2487. Absorption wavelength was set at 220 nm. The mobile phase flow rate was 1.2 mL/min. The injection volume was 100 µL.

6.3.4.3. ZEN

Five g of ground bran sample was mixed with 25 mL of extractant solution (acetonitrile:milli-Q water, 75:25) for 10 min and filtered with Whatman number 1. 10 mL of filtered solution was diluted with 40 mL of PBS and drained through the IAC column. Columns were washed with 20 mL of PBS and ZEN eluted by applying 1.5 mL of HPLC grade acetonitrile and 1.5 ml of milli-Q water, consecutively. Finally, ZEN was determined using the HPLC system described at 2.4.1 and a mobile phase of acetonitrile-water (60:40), adjusted at pH 3.2 with acetic acid. Excitation and emission wavelengths were set at 274 nm and 455 nm, respectively. The mobile phase flow rate was 1 mL/min. The injection volume was 100 µL.

6.3.5. Validation of analytical methods

The analytical methods used were assessed for selectivity, linearity, and precision. Selectivity was checked by injecting 100 µL of mycotoxin standard solution three times before injecting extracted samples and comparing the peak retention times and the fluorescence spectra of the substances that produced these peaks. Standard curves were generated by linear regression of peak areas against concentrations. Precision was established by determining AFB1, AFB2, AFG1, AFG2, OTA, DON and ZEN levels, in wheat bran food samples at least by triplicate, in those samples (blank samples) fortified in order to calculate the recovery rates. The limit of detection (LOD) was considered to be three fold the signal of blank noise, and the limit of quantification (LOQ) was calculated as 3 x LOD. Method performance characteristics for AFs, DON, OTA and ZEN are summarized in Table 6.1. These values are in accordance to performance criteria established by Commission Regulation (EC) Nº 401/2006 (European Commission 2006a).

Table 6.1. Method performance for aflatoxins (AFs), deoxynivalenol (DON), ochratoxin A (OTA) and zearalenone (ZEN) determination in cereal bran.

	LOD[a] (µg/kg)	LOQ[b] (µg/kg)	n	Spiking level (µg/kg)	Recovery (%)[c]	RSDr[d](%)
Aflatoxins						
B_1	0.30	0.90	5	2.5	103 ± 20	20
			5	5.0	89 ± 4	4
			5	7.5	102 ± 29	28
B_2	0.05	0.15	5	2.5	110 ± 15	14
			5	5.0	115 ± 8	7
			5	7.5	109 ± 9	15
G_1	0.10	0.30	5	2.5	110 ± 14	12
			5	5.0	98 ± 4	4
			5	7.5	108 ± 14	13
G_2	0.10	0.30	5	2.5	72 ± 3	4
			5	5.0	70 ± 6	8
			5	7.5	70 ± 12	17
OTA	0.2	0.6	5	2.5	74 ± 16	21
			5	5.0	103 ± 13	13
			5	7.5	95 ±12	13
DON	100	300	5	100	124 ± 8	6
			5	500	86 ± 10	12
			5	1000	99 ± 3	3
ZEN	2	6	5	25	95 ± 5	5
			5	50	98 ± 6	6
			5	100	102 ± 5	5

[a] LOD = Limit of detection.
[b]LOQ = Limit of quantification.
[c] Mean value±standard deviation.
[d] RSDr = relative standard deviation.

6.3.6. Statistical analysis

ANOVA was applied to the whole data matrix in order to detect significant interactions among factors. Kruskal-Wallis test was applied to assess the significance of sample traits in the observed mycotoxin concentration levels. Moreover, Spearman rank correlation test allowed to establish correlations among the observed levels of the different mycotoxins analysed. For data analysis left-censored data were substituted by LOD/2.

6.3.7. Exposure assessment simulation model

For each contamination dataset, the gamma pdf was fitted with the method of maximum likelihood using the CAPABILITY procedure of SAS software (SAS, 2010). The Anderson-Darling statistic was used in the goodness-of-fit test, considering a significance level greater than 95%, to accept the distribution as a suitable candidate. Nine simulation consumption scenarios were taken from 1 to 30 g of fiber per day, normalised to an average individual body weight corresponding to adults of 70 kg.

The stochastic exposure model was adapted from the parametric model reported by Cano-Sancho et al. (2011). Random contamination, drawn from the corresponding adjusted gamma probability density function, was combined with the normalised consumption vector over the simulation set. The mean exposure and other statistics where computed on the histogram built with the simulation of the set (n= 10000).

6.4. Results and discussion

Three from the four mycotoxins (ZEN, DON, AFs and OTA) studied were frequently detected in many samples. AFs were not detected in any sample (LOD 0.55 μg/kg). Only 10 out of 67 samples were free of mycotoxins. The analysed mycotoxin with a major incidence in the samples was DON. It was present in 28 samples (42 %), the range of concentration being between < LOD and 6178 μg/kg. 13 samples (19%) showed levels of DON above the legislative limit in the EU for this kind of products, 750 μg/kg (EC Regulation 1881/2006). ZEN was present in 10 samples of 67 (15%); concentration ranged from < LOD to 25 μg/kg. OTA was present in 17 of 67 samples (25%) and the range of concentration was between < LOD and 2.3 μg/kg.

The predominance of DON in cereals and cereal food products has been reported by other authors (Trigo-Stockeli et al., 1996; Cano-Sancho et al., 2012). DON was the only analyzed mycotoxin that was found in samples at concentration above the EU legal limits for fiber samples. As fungal development in intact grains always starts from the outside of the grain, bran samples usually contain higher concentrations than the whole grains, as it only contains the outer layers

of the seed. Mean mycotoxin concentrations in milled cereal samples found in other studies were 4.8 µg/kg bran of ZEN (maize), and 8.8, 3.4 and 0.5 mg/kg bran of DON (maize, wheat and wheat respectively) (Lauren & Ringrose, 1997, Trigo-Stockli et al., 1996 and Thammawong et al., 2010). However, those studies did not mention the destination of the milling fractions analysed, either animal or human consumption, while in our case samples were final products intended only for direct human consumption.

6.4.1. Differences in mycotoxin levels due to type of cereal

From the 67 samples analyzed, 37 belonged to wheat bran and 30 to oat bran categories (Table 6.2). Kruskal-Wallis test revealed significant differences in the levels of DON ($p < 0.05$); no significant interactions were observed with other factors. Sixty-two percent of wheat samples were contaminated by DON (1308 µg/kg of mean concentration for positive samples), while 17 % of oat samples contained DON (mean concentration for positive samples was 230 µg/kg). OTA was detected in 30 and 20 % of wheat and oat bran samples, respectively, with a mean level of 1.1 (wheat) and 0.3 (oat) µg/kg. ZEN was present in 13 and 17 % of wheat and oat samples, respectively, with mean levels between 8 (wheat) and 8 (oat) µg/kg.

In summary, oat samples showed less concentration of DON than wheat samples. Higher concentration of DON and ZEN in wheat samples has been reported by Martos et al. (2010). As a result, exposure to DON through dietary fiber would be lower when bran oat is the source of such fiber.

Table 6.2. Occurrence of zearalenone (ZEN), deoxynivalenol (DON) and ochratoxin A (OTA) in fiber samples, based on the type of cereal (µg/kg).

	n [a]	ZEN					DON					OTA				
		% [b]	M [c]	SD [d]	Max [e]	m [f]	%	M	SD	Max	m	%	M	SD	Max	m
Wheat	37	13	8	8	21	4	62	1308	1463	6178	825	30	1.1	0.8	2.3	0.9
Oat	30	17	8	9	25	4	17	230	34	276	230	20	0.3	0.1	0.4	0.3

[a] n, total number of analysed samples (% of samples >LOD)
[b] Percentage of samples >LOD
[c] Mean of positive samples (>LOD)
[d] Standard deviation of positive samples (>LOD)
[e] Maximum concentration.
[f] Median of positive samples (>LOD).

6.4.2. Differences in mycotoxin levels due to thermal processing treatment

Some of the fiber samples are sold not as raw fiber but as processed, which primarily involves the application of heat or thermo-mechanical treatment. Thus, there were samples which had been subjected to toasting or extrusion. The results have shown some differences in the levels of OTA in the samples ($p < 0.05$). No significant interactions were observed with the remaining factors. Although the percentage of contaminated samples was higher in the heat treated group, the mean levels of contamination were significantly lower in the treated group compared to the unheated (0.4 µg/kg vs 0.9 µg/kg, for the OTA positive samples) (Table 6.3). Despite the relative thermostability of mycotoxins, some studies have shown that ZEN , DON, AFs and OTA could be affected to some extent by heat treatment of cereals and investigation on the effect of heat treatment on called mycotoxins have been done (Scudamore et al., 2008; Neira et al., 1997; Valle-Algarra et al., 2009; Saalia and Philips, 2011; Castells et al., 2006; Boudra et al., 1995).

ZEN was also more often detected in treated samples (18 vs 14%) but, although not significant, the ZEN level was higher in the untreated samples. Some studies show low decreases in ZEN concentration in the samples which are subjected to heat treatments (Ryu et al. 2002; Ryu et al, 1999 and Scudamore et al., 2008), and they conclude that higher temperatures are required for reduction of ZEN concentration. ZEN concentration in treated and untreated samples were not statistically different ($p > 0.05$).

Finally, samples which were not subjected to a heat treatment were less often contaminated by DON and with lower levels, although the difference was not significant ($p>0.05$) (Table 6.3). The temperature for DON degradation has been widely studied linked to extrusion cooking. However, contradictory conclusions are reached ranging from no changes in DON concentration to complete loss (Cazzaniga et al., 2001; Scudamore et al., 2008).

In summary, in this study only OTA have demonstrated lower levels in the samples which suffered a heat treatment.

Table 6.3. Occurrence of zearalenone (ZEN), deoxynivalenol (DON) and ochratoxin A (OTA) in fiber samples, based on the presence of a heat treatment (µg/kg).

	n [a]	ZEN					DON					OTA				
		% [b]	M [c]	SD [d]	Max [e]	m [f]	%	M	SD	Max	m	%	M	SD	Max	m
No heat treatment	56	14	9	9	25	4	37	981	991	3079	789	20	0.9	0.8	2.3	0.4
Heat treatment	11	18	3	1	4	3	64	1520	2260	6178	448	45	0.4	0.0	0.4	0.4

[a] n, total number of analysed samples
[b] Percentage of samples >LOD
[c] Mean of positive samples (>LOD)

[d] Standard deviation of positive samples (>LOD)
[e] Maximum concentration.
[f] Median of positive samples (>LOD).

6.4.3. Differences in mycotoxin levels due to type of production (organic vs conventional)

There were 26 samples from organic production and 41 samples from conventional production. Kruskal-Wallis test revealed that there was significant differences in the levels of OTA in wheat bran samples ($p < 0.05$), but not in oat bran ones. No significant differences were found for DON and ZEN in any of the cereal brans. Table 6.4 shows the results for wheat bran samples. 45 % of organic wheat samples contained OTA while only 15 % of the conventional samples were positive; moreover, the mean OTA level of positive samples was 1.4 µg/kg in the organic samples compared to 0.6 µg/kg in the conventional ones. Our investigation shows higher OTA levels in the organic than in the conventional production, as in other previous reports (Leifert and Cooper, 2008; Anselme et al. 2006).

For ZEN, a major percentage of contaminated samples were observed in the conventional group, although mean concentration levels were higher in the organic group, while for DON it was just the opposite situation (although differences were not significant in any of the cases). Some reports show differences between conventional/organic productions in the Fusarium mycotoxins, with lower incidence of Fusarium contamination and Fusarium mycotoxins in the organic cereals than in the conventional ones (Bernhoft et al., 2010; Klinglamyr et al., 2010).

Table 6.4. Occurrence of zearalenone (ZEN), deoxynivalenol (DON), ochratoxin A (OTA) in fiber samples based on the type of production in the wheat fiber samples (µg/kg).

	n [a]	ZEN					DON					OTA				
		%[b]	M[c]	SD[d]	Max[e]	m[f]	%	M	SD	Max	m	%	M	SD	Max	m
Organic	11	9	21	0	21	21	72	655	565	1662	426	45	1.4	1.0	2.3	1.6
Conventional	26	15	4	3	9	3	58	1657	1681	6178	923	15	0.6	0.3	1.0	0.4

[a] n, total number of analysed samples.
[b] Percentage of samples >LOD.
[c] Mean of positive samples (>LOD).
[d] Standard deviation of positive samples (>LOD).
[e] Maximum concentration.
[f] Median of positive samples (>LOD).

6.4.4. Differences in mycotoxin levels due to other factors

Other factors have been analyzed (city of sample purchase, type of presentation, presence of other ingredients…), but none of them showed any effect in the analyzed mycotoxins.

The samples were purchased in two different cities (Lleida and Valencia), thus consumers from both cities are expected to be equally exposed to ZEN , DON and OTA through wheat and oat bran consumption. Globalization of the market tends to diminish the impact of geographical differences in food consumption.

The presence of other ingredients (sugar, maize, honey, salt, egg …) in the samples did not either increase or reduce the mycotoxin presence in the analyzed samples, as they were present in small percentages.

6.4.5. Co-occurrence

Co-occurrence of 2 or 3 different mycotoxins was observed in 18 % of samples (Table 6.5), although no samples with the four studied mycotoxins were detected. A positive correlation between ZEN and DON content in the samples ($p < 0.05$) was observed. In 90 % of the samples where ZEN was detected, DON was also present. The two mycotoxins are produced by Fusarium and their co-occurrence in cereals has been reported in other studies (Stanković et al., 2012; Lee et al., 2011).

No significant correlation was observed among the remaining mycotoxins.

Table 6.5. Number and percentage of samples with mycotoxin co-occurrence.

	Two mycotoxins						Three mycotoxins			
	ZEN x DON	ZEN x AFs	ZEN x OTA	DON x AFs	DON x OTA	AFs x OTA	ZEN x DON x AFs	ZEN x DON x OTA	DON x OTA x AFs	ZEN x AFs x OTA
Number samples (%)	7 (10.5)	0 (0.0)	3 (4.5)	0 (0.0)	7 (10.5)	0 (0.0)	0 (0.0)	3 (4.5)	0 (0.0)	0 (0.0)

6.4.6. Exposure assessment

The low presence of ZEN and OTA in bran did not permit us to carry out an accurate exposure assessment simulation model; therefore we excluded these mycotoxins from the

statistics. The DON contamination dataset was used in the calculations with previous substitution of left-censored data by LOD/2.

Results of exposure to DON considering the different consumption scenarios are shown in the Table 6.6. The range considered in the model was between 1 and 30 g/day, and the mean DON intake from bran was expected between 0.006 and 0.208 µg/kg bw/day. Highest estimations were found for the percentile 95 with extreme values, reaching estimations of 0.800 µg/kg bw/day.

Table 6.6. Estimated deoxynivalenol (DON) exposure from different fiber intake scenarios (from 1 to 30 g/day) for one adult (70 kg) computed with the raw contamination datasets (10000 simulations).

Fiber intake	**Fiber intake (70 kg)**	***Mean***	***sd***	***p50***	***p75***	***p90***	***p95***
g/day	***g/kg bw/day***	***µg DON kg bw day***					
1	0.014	0.006	0.009	0.003	0.009	0.018	0.026
2.5	0.036	0.017	0.024	0.008	0.023	0.048	0.067
5	0.071	0.034	0.047	0.016	0.046	0.094	0.132
7.5	0.107	0.052	0.072	0.024	0.069	0.142	0.199
10	0.143	0.069	0.096	0.032	0.092	0.190	0.266
15	0.214	0.104	0.144	0.048	0.138	0.285	0.399
20	0.286	0.139	0.193	0.064	0.185	0.381	0.533
25	0.357	0.173	0.240	0.080	0.231	0.475	0.666
30	0.429	0.208	0.289	0.097	0.278	0.571	0.800

In order to assess the expected contribution of DON exposure through dietary bran consumption to the global exposure of Catalonian population, our estimations were added to the estimations for adults given in Cano-Sancho et al. (2011). The median exposure values of 0.22 and 0.32 µg/kg bw/day were considered in the statistics, for adult females and males, respectively (results shown in Table 6.7).

Table 6.7. Relative contribution of fiber intake to the global exposure of Catalonian population to deoxynivalenol (DON). Median exposure values of 0.22 and 0.32 µg/kg bw/day for males and females, were considered, respectively (from Cano-Sancho et al. 2011).

Fiber intake scenarios	*Percentile 50*					*Percentile 90*				
	Males			Females		Males			Females	
	DIF	DGE	*C*	DGE	*C*	DIF	DGE	*C*	DGE	*C*
g/day	µg/kg bw/day	µg/kg bw/day	*%*	µg/kg bw/day	*%*	µg/kg bw/day	µg/kg bw/day	*%*	µg/kg bw/day	*%*
1	0.003	0.223	1.4	0.323	1.0	0.019	0.239	7.8	0.339	5.5
2.5	0.008	0.228	3.6	0.328	2.5	0.048	0.268	17.9	0.368	13.0
5	0.016	0.236	6.8	0.336	4.8	0.095	0.315	30.1	0.415	22.8
7.5	0.024	0.244	9.9	0.344	7.1	0.143	0.363	39.3	0.463	30.8
10	0.032	0.252	12.8	0.352	9.2	0.191	0.411	46.4	0.511	37.3
15	0.049	0.269	18.1	0.369	13.2	0.285	0.505	56.5	0.605	47.1
20	0.065	0.285	22.8	0.385	16.9	0.381	0.601	63.4	0.701	54.4
25	0.081	0.301	26.9	0.401	20.2	0.476	0.696	68.4	0.796	59.8
30	0.097	0.317	30.7	0.417	23.3	0.572	0.792	72.2	0.892	64.1

DIF, Deoxynivalenol exposure from fiber supplement intake (units in µg/kg bw/day).
DGE, Deoxynivalenol global exposure considering other sources of exposure (median values) from Cano-Sancho et al. (2011) (units in µg/kg bw/day).
C, Relative contribution of fiber supplement intake to the global exposure to DON (%)

Our results showed a great contribution of fiber supplements to the global exposure of Catalonian adults to DON. Most of fibre supplements are offered in supermarkets and health food stores to reach the dietary recommendations of fiber (between 25 and 30 g/day) because low fibre intake

is commonly provided by the current diet in Spain (16.5 g/day). Based on a consumption of 5 g fiber/day (common recommendation in several diets), we estimated a contribution between 4.8 and 6.8 % for the percentile 50, this range being 22.8-30.1 % in case of percentile 90, depending of gender (female/male). Therefore, fiber supplements intake can be an important source of DON, thus it is important to include this category in global exposure assessment studies of this mycotoxin.

6.5. References

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