Overview

- **96 % of breakfast cereals and 90 % of cereal based baby foods analyzed presented mycotoxins;**
- **92 % of breakfast cereals and 60 % of cereal based baby foods revealed co-ocurrence of mycotoxins;**
- **2 to 7 mycotoxins were found simultaneously in the same sample;**
- **32 different combinations of mycotoxins were found in all analyzed samples.**

Assessment of mixtures of mycotoxins in cereal based foods available in Portuguese market

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Introduction

Mycotoxins are secondary metabolites of fungi that cause toxic and carcinogenic outcomes in humans and animals exposed to them[1]. These toxins affect several commodities including cereal grains and their finished products, infant formula and baby foods[2]. Cereals play a vital role in diet because they are not only providing humans with essential macronutrients for growth and maintenance but also supply other important vitamins, minerals, and micronutrients for optimal health[3]. Processed cereal samples that can be mixtures of different raw materials, are prone to contain various types of mycotoxins. A previous Portuguese study of the children food consumption revealed that about 50 % of children aged between 12 and 36 months consumes breakfast cereals[4]. This study, performed under the Mycomix project (PTDC/DTP-FTO/0417/2012), aims to determine the incidence and levels of 21 mycotoxins and metabolites (AFB_1, AFB_2, AFG_1, AFG_2, AFM_1, OTA, NIV, NEO, DAS, FUS-X, DON, 15-ADON, 3-ADON, HT-2, T-2, VER, T-2 TETROL, T-2 TRIOL, FB_1, FB_2 and ZEA), in breakfast cereals and cereal based baby foods, available in the Portuguese market.

Material and Methods

26 breakfast cereal samples:

-Wheat, Maize, Oat, Rice, Multigrain, Chocolate;

-Breakfast cereals primarily marketed for children;

-1 Kg each sample;[5]

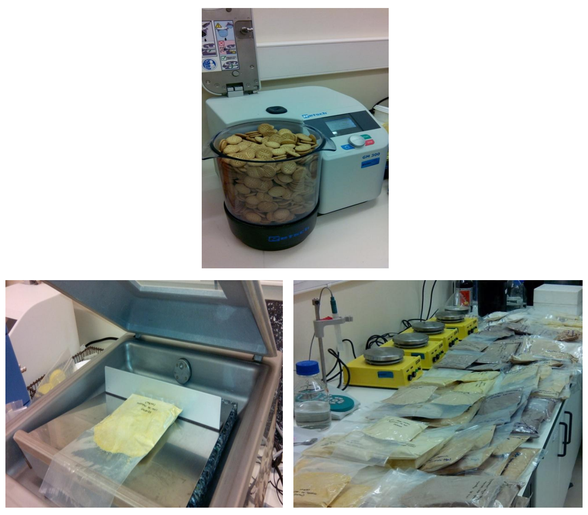
-Purchased in Lisbon, in 2014.

20 cereal based baby food samples:

-Wheat, Maize, Rice, Multigrain;

- 1 Kg each sample;[5]

-Purchased in Lisbon, in 2015.



Extraction with Methanol:Water (80:20) Purification with IAC VICAM® AflaOchra HPLC-FLD, with Kobra-cell derivatization	AFM_1, AFB_1, AFB_2, AFG_1, AFG_2, OTA
Extraction with Methanol:Water (75:25) Centrifugation, dilution and injection Internal Standard - $^{13}C_{18}$-ZEA UPLC-MS/MS	ZEA
Extraction with Methanol:Water (75:25) Centrifugation, dilution and injection UPLC-MS/MS	FB_1, FB_2
Extraction with QuEChERS Purification with dispersive SPE Derivatization: BSA+TMCS+TMSI GC-MS	DON, NIV, NEO, DAS, FUS-X, VER, T-2 TETROL, T-2 TRIOL 15-ADON, 3-ADON, T-2, HT-2

Results and Discussion

Table 1 – Method perfomance for the determination of the analyzed mycotoxins by HPLC-FLD, UHPLC-MS/MS and GC-MS/MS.

	AFM_1	AFB_1	AFB_2	AFG_1	AFG_2	OTA
Linearity (µg kg^{-1})	0.100 – 1.000	0.040 – 0.400	0.030 – 0.300	0.045 – 0.450	0.030 – 0.300	0.200 – 2.000
LOD (µg kg^{-1})	0.011	0.003	0.001	0.006	0.010	0.006
LOQ (µg kg^{-1})	0.032	0.009	0.004	0.018	0.029	0.019
Recovery (%)	83	73	57	87	57	71

	FB_1	FB_2	ZEA
Linearity (µg kg^{-1})	2.5 - 800	2.5 - 800	0.24 – 10.00
LOD (µg kg^{-1})	0.06	0.12	0.12
LOQ (µg kg^{-1})	0.18	0.36	0.40
Recovery (%)	70	68	*

	DON	NIV	T-2	HT-2	NEO	DAS	FUS-X	15-ADON	3-ADON	VER	T2-Tetrol	T-2 Triol
Linearity (µg kg^{-1})	15 - 360	25 - 360	25 - 360	25 - 450	15 - 360	15 - 360	15 - 360	15 - 360	75 - 360	75 - 360	35 - 450	15 - 360
LOD (µg kg^{-1})	0.4	5.6	6.8	6.4	1.3	3.1	2.8	2.5	17.3	19.2	10.5	0.9
LOQ (µg kg^{-1})	1.2	18.4	22.3	21.1	4.2	10.1	9.2	8.3	57.0	63.3	34.6	3.0
Recovery (%)	93	46	44	93	103	117	99	131	92	135	84	74

* Internal standard $^{13}C_{18}$-ZEA used. Recovery (%) not determined.

All these results, with the exception of nivalenol and T2 recoveries, were in agreement with the criteria mentioned in the Commission Regulation (EC) No. 401/2006 and showed that the analytical methods applied are adequate for mycotoxins determinations.

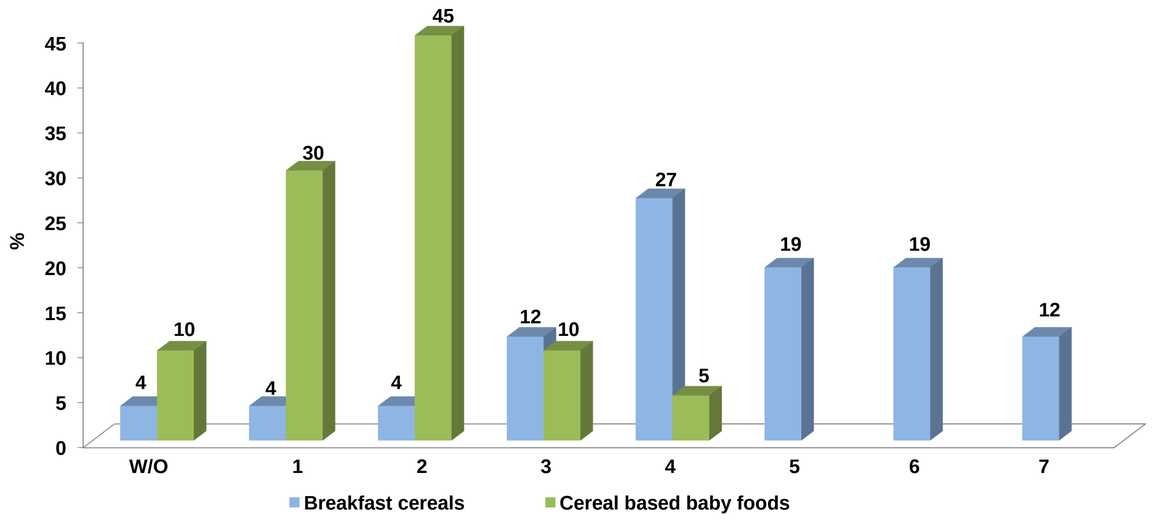


Figure 1 – Co-occurrence of mycotoxins in breakfast cereal and cereal based baby food samples. The X-axis represents the number of mycotoxins determined in the same sample (W/O = no mycotoxins detected) and the Y-axis the respective percentage.

Co-occurrence of mycotoxins (2 to 7, simultaneously) was highly observed in the analyzed samples:

92 % in breakfast cereals and 60 % in cereal based baby foods.

Table 2 - Occurrence of mycotoxins (µg kg^{-1}) in 26 breakfast cereal samples and 20 cereal based baby foods, purchased in supermarkets in Lisbon region. AFG_2, NEO, DAS, FUS-X, DON, 15-ADON, 3-ADON, HT-2, T-2, VER, T-2 TETROL, T-2 TRIOL were not detected in breakfast cereal samples. DON, NIV, NEO, DAS, FUS-X, DON, 15-ADON, 3-ADON, HT-2, T-2, VER, T-2 TETROL, T-2 TRIOL analysis in cereal based baby foods are still in course.

		AFM_1	AFB_1	AFB_2	AFG_1	AFG_2	OTA	FB_1	FB_2	DON	NIV	ZEA
Breakfast cereals	> LOD (n)	3	19	7	1	0	18	15	10	16	1	19
	> LOD (%)	12	73	27	4	0	69	58	38	62	4	73
	Maximum (µg Kg^{-1})	**0.024**	**0.130**	**0.011**	**0.014**	**ND**	**0.100**	**67.0**	**14.0**	**207.8**	**27.1**	**5.61**
Cereal based baby foods	> LOD (n)	8	0	1	2	0	10	7	0	*	*	3
	> LOD (%)	40	0	5	10	0	50	35	0	*	*	15
	Maximum (µg Kg^{-1})	**0.190**	**ND**	**0.002**	**0.016**	**ND**	**0.263**	**0.860**	**ND**	*****	*	**0.98**

* Analysis still in course. ND = Not detected.

- All samples presented levels below the maximum limits established by the Commission Regulation 1881/2006[6], when available.
- 96 % of the breakfast cereals samples and 90 % of the cereal based baby food samples were contaminated with mycotoxins (values > LOD).
- Cereal based baby foods with milk presented a maximum value of 0.190 µg AFM_1 kg^{-1}. There is no legislation for the presence of AFM_1 in cereal based baby foods and the maximum admissible level for AFM_1 in infant formulae is 0.020 µg kg^{-1}.
- Breakfast cereals presented a maximum value of 0.130 µg AFB_1 kg^{-1}. There is also no legislation for the presence of AFB_1 in breakfast cereals and it should be noted that the maximum levels admissible for AFB_1 in cereal based baby foods is 0.100 µg kg^{-1}.
- ZEA, AFB_1, OTA, DON and FB_1 were the most commonly detected mycotoxins in breakfast cereals, ranging between 58 % and 73 % of samples with contents above LOD.
- OTA, AFM_1 and FB_1 were the most commonly detected mycotoxins in cereal based baby foods with 50 %, 40 % and 35 % of the samples with contents above LOD, respectively.

Breakfast Cereals

22 different combinations of mycotoxins

The most frequent were:

AFB_1+AFB_2+OTA+ZEA (n=2) and

OTA+FB_1+DON+ZEA (n=2)

Cereal based baby foods

10 different combinations of mycotoxins

The most frequent was:

OTA+FB_1 (n=3)

Conclusions

The present study evaluated for the first time the occurrence of mycotoxins mixtures in breakfast cereals and cereal based baby foods marketed in Portugal, with the analysis of 21 mycotoxins and their metabolites. These results contribute to increase the knowledge on mycotoxin contents in these food matrix and highlight an urgent need for further studies in order to overcome the absence of legislated limits for mycotoxins in breakfast cereals other than DON and FB_1 and also the absence of legislated limits for mycotoxin mixtures in food. The last issue assumes particular importance considering the potential interactions that could occur between mycotoxins and its potential impact on human and, mainly, children health.

Acknowledgments: This research was supported by the MYCOMIX project (PTDC/DTP-FTO/0417/2012, FCT, Portugal).

Poster presented on RAFA 2015 (3-6 November), Prague, Czech Republic .

[1]Wu et al (2014) Annual Reviews of Food Science and Technology 5:351-372; [2]Turner et al (2012) Nutrition Research Reviews 25:162-179; [3]Collins et al (2010) Cereal Chemistry 87(4) 272–282; [4]Presentation "Alimentação e crescimento nos primeiros anos de vida: a propósito do EPACI Portugal 2012", Lisbon, 01/11/2013; [5]Commission Regulation (EC) Nº 401/2006 of 23 February 2006 ; [6]Commission Regulation (EC) Nº 1881/2006 of 23 February 2006;