Bioaccessibility assessment of patulin and ochratoxin A in cereal and fruit based baby foods using a harmonized *in vitro* digestion model – contribution for the risk assessment of chemical mixtures

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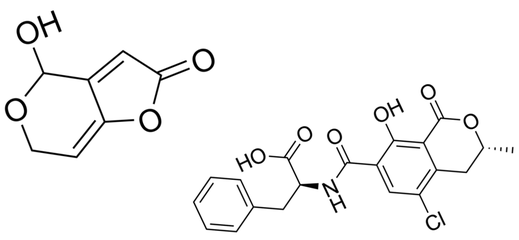
Background & Aims

The total amount of an ingested contaminant (external dose) does not always reflect the amount that is available to the body (internal dose)[1]. **Mycotoxins** are fungal natural contaminants commonly found in a variety of foods including baby foods and have been found in cereal and fruit based baby foods. The amount of mycotoxin resisting to the digestion process and potentially absorbable by the systemic circulation and consequently producing it toxic effects – **bioaccessibility** – is only a part of that ingested.

This study aimed to evaluate the

1) **bioaccessibility** of **Ochratoxin A (OTA) and Patulin (PAT) in cereal and fruit based baby food**;
2) effect of the **presence/absence of fruit** in the bioaccessibility of PAT and OTA;
3) possible **interactions** that could happen when these mycotoxins co-occur.



Materials & Methods

• **Samples:**

Cereal (n=3) and cereal and fruit (n=3) based baby food samples were artificially contaminated to 20 µg Kg^{-1} of PAT, 1 µg Kg^{-1} OTA or their mixture (PAT+OTA)

• ***In vitro* Digestion method:**

Bioaccessibility studies were performed according to the harmonized IVD model described by Minekus *et al.* (2014)[2]. This model resulted from an international consensus concerning several aspects as fluids composition, enzymatic activities and sample and fluids amount (Fig. 1).

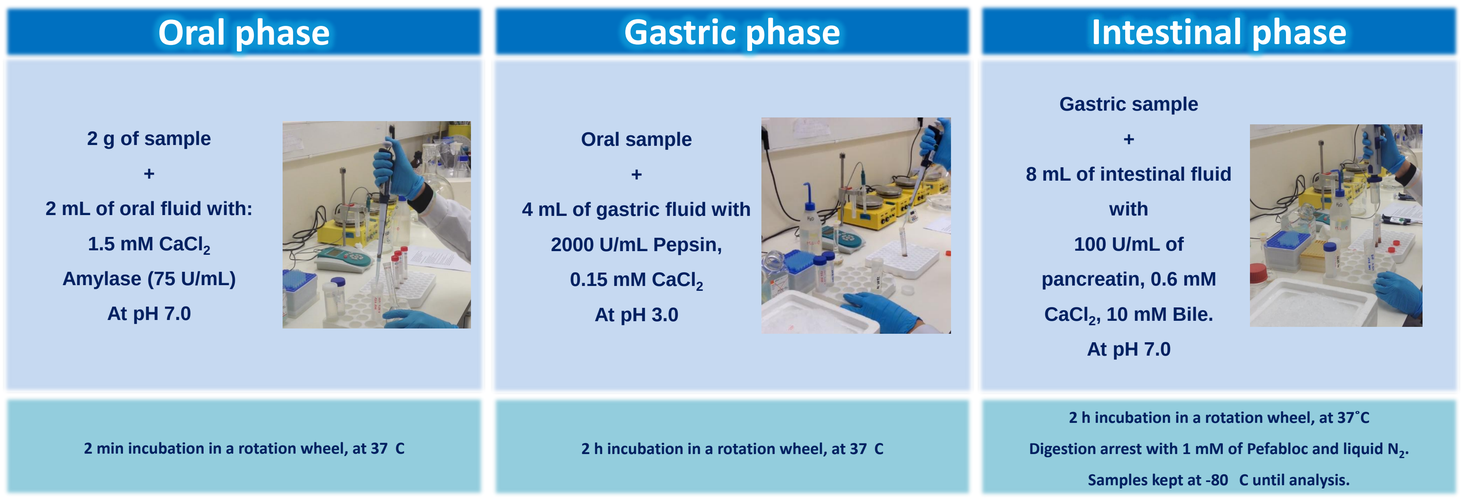


Figure 1: *In vitro* digestion procedure according to Minekus et al., 2014[2].

• **Extraction & analysis methods:**

PAT extraction with n-hexan, ethyl acetate; Purification with SPE; Quantification by HPLC-UV

OTA extraction with MeOH/H_2O (80:20); Purification with Immunoaffinity Columns (IAC); Quantification by HPLC-FLD with Kobra-cell.

• **Matrix interferences in HPLC analysis**

To evaluate possible matrix interferences in HPLC analysis, blank samples were digested and then contaminated at same mycotoxins concentration, and recovery values were determined.

Results

Table 1: Bioaccessibility (%) results of PAT and OTA in cereal and fruit based baby food samples (n=6), artificially contaminated. Bioaccessibility values are expressed as mean ± SE of three replicates. "F" and "W/o" samples represent samples with and without fruit, respectively.

	Patulin					Ochratoxin A				
	Single		Mixture		Recovery from Matrix (%)	Single		Mixture		Recovery from Matrix (%)
F1	70	3.2	54	0.9	105	95	0.3	104	1.2	55
F2	42	1.2	69	4.3	99	105	1.5	103	1.2	65
F3	56	1.8	47	4.2	125	97	1.8	107	0.7	74
W/o 1	77	1.9	64	1.5	125	98	1.5	109	0.1	65
W/o 2	39	0.7	30	1.9	119	102	0.3	107	1.5	72
W/o 3	30	2.5	61	0.6	113	102	3.9	108	0.6	65

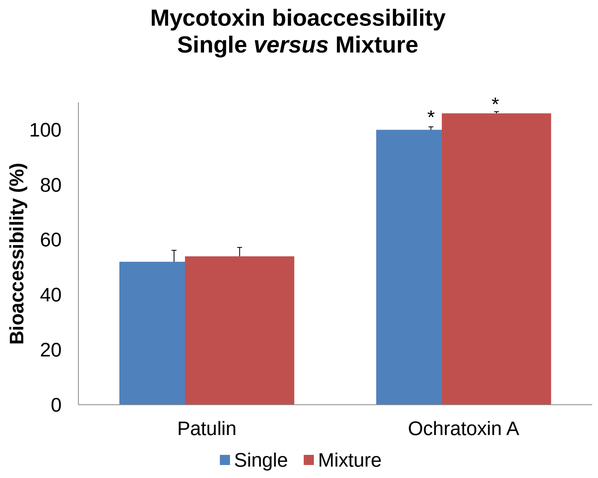


Figure 2: Bioaccessibility (%) results of PAT and OTA in cereal and fruit based baby food samples (n=6), considering the presence of single or mixture of mycotoxins. Statistically significant differences were verified when compared single and mixture OTA bioaccessibility results (*, Wilcoxon Signed Rank test, p=0.001). No significant differences were found when comparing single and mixture PAT bioaccessibility results (Wilcoxon Signed Rank test, p=0.777).

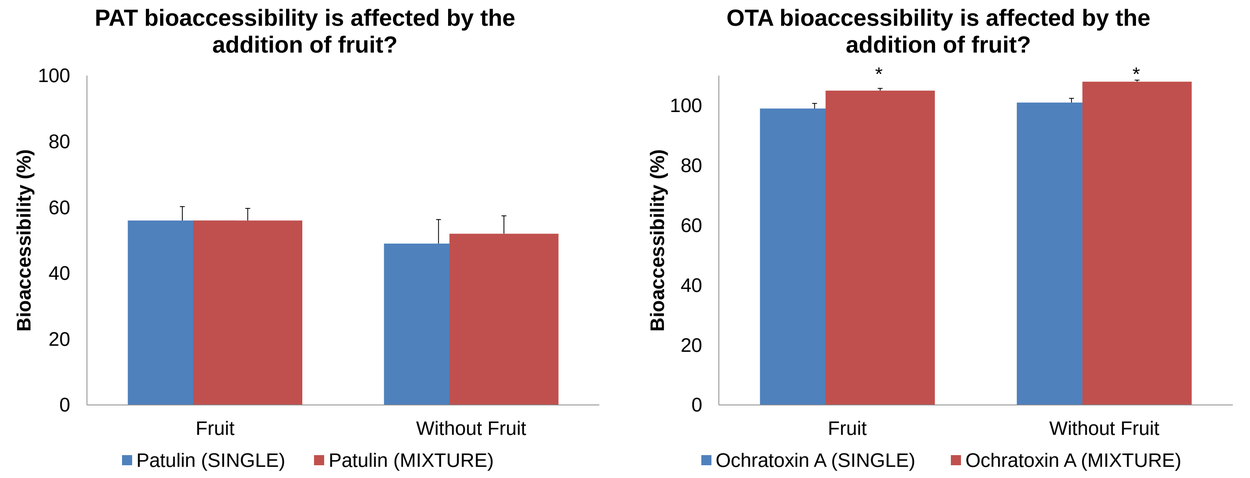


Figure 3: Bioaccessibility (%) results of PAT and OTA in cereal and fruit based baby food samples (n=6) and its relationship with the presence of fruit. Statistically significant differences were determined when compared OTA in mixture, considering samples with and without fruit (*, Mann-Whitney test with p=0.02). For the remaining test scenarios no significant differences were found [PAT, single (p=0.222) and mixture (p=0.863), and OTA, single (p=0.489)].

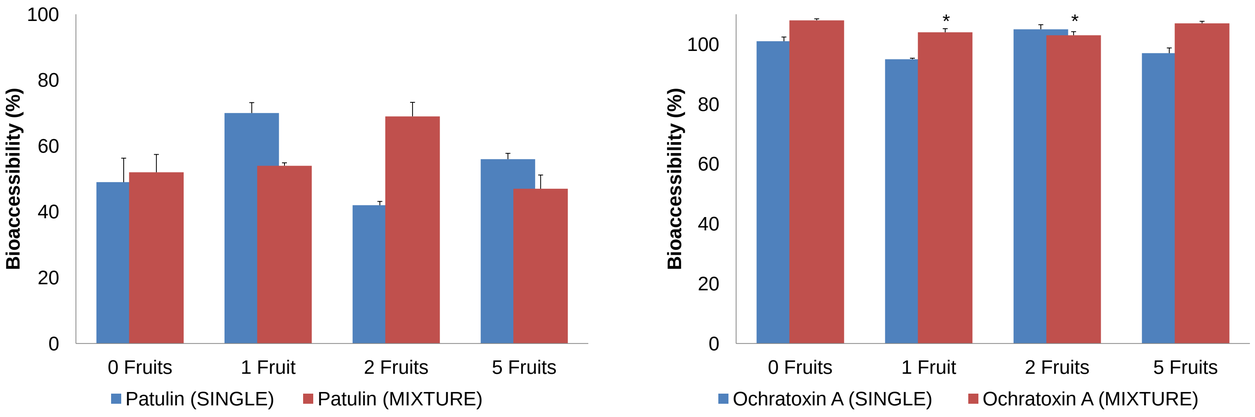


Figure 4: Bioaccessibility (%) results of PAT and OTA in cereal and fruit based baby food samples (n=6) and its relationship with the number of fruits present in baby foods. Comparison were made between samples without fruits and samples with 1, 2 and 5 fruits. Statistically significant differences were determined for 1 and 2 fruits, comparing to 0 fruits, for OTA in mixture (*, Mann-Whitney test with p=0.018 and p=0.009, respectively). For the remaining test scenarios no significant differences were found.

Highlights:

1. A **significant portion of PAT and OTA can reach the small intestine** and thus, be available to cross the intestinal barrier and produce their toxic effects.
2. **Simultaneous presence of mycotoxins** affects the **bioaccessibility** values. Further studies with different mixtures of mycotoxins are needed to corroborate it.
3. The presence/absence of **fruit** and the **number of fruits** in cereal and fruit based baby foods indicated a potencial influence in **mycotoxins bioaccessibility**.

This study provides new information related with the bioaccessibility of mycotoxins in baby foods. The results offer a more comprehensive picture of what occurs during the digestion of food contaminants in the gastrointestinal tract and subsequently contribute to provide a more accurate risk assessment of single and multiple food contaminants.

References: [1]Versantvoort, C., Oomen, A., Van de Kamp, E., Rompelberg, C., Sips, A. (2005). *Food Chem. Toxicol.*, *43*, 31-40; [2]Minekus *et al.* (2014). *Food & Function*, 5: 1113-1124

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