Food & Function

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Cite this: *Food Funct.*, 2012, **3**, 853

www.rsc.org/foodfunction

PAPER

The soluble dietary fiber inulin can influence the bioaccessibility of enniatins

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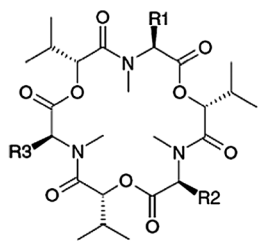
Received 16th August 2011, Accepted 21st April 2012
DOI: 10.1039/c2fo00004k

Enniatins (ENs) are bioactive compounds produced by the secondary metabolism of several *Fusarium* strains and are known to have various biological activities, such as acting as enzyme inhibitors, antifungal antibacterial agents, and immunomodulatory substances. This study investigated the bioaccessibility of the ENs in wheat crispy breads produced with three different inulin concentrations (1, 5 and 10%). The mean bioaccessibility data of the four ENs (A, A_1, B and B_1) ranged from 68.67% to 84.67 in the experiments carried out without inulin, whereas the data ranged from 51.00 to 74.00% in the experiments carried out with the wheat crispy bread produced with 5 and 10% of the inulin.

Introduction

The enniatins (ENs) are secondary fungal metabolites that have been known for several decades.[1] They are six-membered cyclic depsipeptides, which are commonly composed of three D-a-hydroxyisovaleric acid (Hiv) residues linked alternatively to three L-configured N-methyl amino acid residues to give an 18-membered cyclic skeleton (Fig. 1).[2] ENs are produced by strains of some species of fungal genera *Alternaria*, *Fusarium*, *Halosarpheia* and *Verticillium*.[3] The ENs normally produced by *Fusarium subglutinans*, *Fusarium proliferatum* and *Fusarium tricinctum* strains[4] are contaminants of cereals, especially maize and its derivative.



Enniatin	R1	R2	R3
A	$CH(CH_3)CH_2CH_3$	$CH(CH_3)CH_2CH_3$	$CH(CH_3)CH_2CH_3$
A_1	$CH(CH_3)CH_2CH_3$	$CH(CH_3)CH_2CH_3$	$CH(CH_3)_2$
B	$CH(CH_3)_2$	$CH(CH_3)_2$	$CH(CH_3)_2$
B_1	$CH(CH_3)_2$	$CH(CH_3)_2$	$CH(CH_3)CH_2CH_3$

Fig. 1 Chemical structure of ENs.

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Besides their antibiotic activity, ENs inhibit the enzyme acyl-CoA:cholesterol acyltransferase (ACAT).[5] They are also known as phytotoxins and are associated with plant diseases characterized by wilt and necrosis.[1]

Different studies have indicated that ENs, change the monovalent ion transport across membranes and disrupt the ionic selectivity of cell walls. This effect is particularly debilitating in mitochondrial membranes, resulting in the uncoupling of oxidative phosphorylation.[5]

A new model of vertically stacked enniatin molecules that form sandwich complexes in cell membranes was suggested.[6] Mixtures of ENs or individual compounds have been shown to possess substantial cytotoxicity.[7]

ENs were shown to inhibit one of the major multidrug efflux pumps (Pdr5p in *Saccharomyces cerevisiae*) at nontoxic levels *via* a different mechanism.[8,22,23] As such, in combination with chemotherapeutic drugs, their pharmacological properties have the potential for many important clinical applications. ENs show a great efficiency to inhibit cell growth in several human carcinoma cell lines at the micromolar level.[9,24,25,27]

To achieve any effects in a specific tissue or organ, the mycotoxins have be available, which refers to the compound's tendency to be extracted from the food matrix, and they must then be absorbed from the gut *via* the intestinal cells. The metabolic routes for ENs contained in food and their metabolites in humans are summarized in the Fig. 2.

The term bioavailability has several working definitions, depending on the research area where it applies to. From the nutritional point of view, bioavailability refers to the fraction of the nutrient or bioactive compound ingested that is available for use in physiologic functions or to be stored.[10]

In 1953, nutritionist E. H. Hipsley introduced the term "dietary fiber" to represent intake of the indigestible components of plant cell walls.[11] According to the American Association of Cereal Chemists (AACC) Dietary Fiber Definition Committee, dietary fiber is defined as the edible parts of plants or analogous

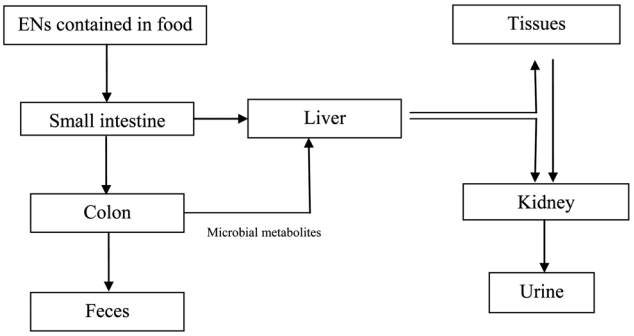

Fig. 2 Routes for ENs contained in food and their metabolites in humans.

carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine.

Dietary fibers may be classified as water-soluble or gel-forming viscous fibers and water insoluble fibers.[12] Insoluble fibers consist mainly of cell wall components such as cellulose, lignin, and hemicelluloses present mainly in wheat, most grain products, and vegetables. Soluble fiber consists of noncellulosic polysaccharide such as pectin, gums and mucilage found in fruits, oat, barley, dried beans, and legumes.[13]

Soluble dietary fibers are highly fermentable and are associated with carbohydrate and lipid metabolism and have been shown to exhibit hypocholesterolemic properties,[14] while, insoluble fibers contribute to fecal bulk and transit times and have little or no effect on cholesterol metabolism.

Inulin is a term applied to a heterogeneous blend of fructose polymers found widely distributed in nature as plant storage carbohydrates; it is a functional food ingredient that provides unique combination of technological properties. Furthermore, it provides nutritional benefits, which results in better health and attenuation of the risk of many diseases.[15] The high solubility of inulin enables it to be fortified in dairy products such as milk drinks, yogurt, cheeses, and desserts.[16]

Several animal and human studies have shown that inulin functions as a prebiotic, in promoting good digestive health, influencing lipid metabolism and decreasing risk of osteoporosis by increasing calcium absorption.[14] Inulin is also found to exhibit some effect on insulinaemia and glycaemia, as well as reduce the risk of colon cancer, breast cancer, and tumour growth.[17]

The aim of this work was to study the bioaccessibility of ENs **A**, $\mathbf{A_1}$, **B** and $\mathbf{B_1}$ (Fig. 1) spiked in a concentration of 1.5 and 3.0 μM in wheat crispy breads, produced with three different inulin concentrations.

Materials and methods

Materials

Potassium chloride (KCl), potassium thiocyanate (KSCN), monosodium phosphate (NaH_2PO_4), sodium sulphate ($NaSO_4$), sodium chloride (NaCl), sodium bicarbonate ($NaHCO_3$), urea, α-amilase, hydrochloric acid (HCl), pepsin, pancreatin, bile salts and inulin were obtained from Sigma-Aldrich (Madrid, Spain). Acetonitrile, methanol and ethyl acetate were purchased from Fisher Scientific (Madrid, Spain). Deionized water (<8 MΩ cm resistivity) was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA). Chromatographic solvents and water were degassed for 20 min using a Branson 5200 (Branson Ultrasonic Corp., CT, USA) ultrasonic bath.

The ENs (A, A_1, B, B_1), used in this study were produced and purified (>98%) according to the method of Meca *et al.*[18]

Wheat crispy bread production

For the production of the wheat crispy bread with different inulin concentrations, 300 g of wheat flour was mixed with 180 mL of water, 3 g of sucrose and 6 g of NaCl. This mixture was shook for 5 min, and then the dough obtained was divided into three parts. Each of the three dough parts was enriched with a specified amount of inulin (1, 5, or 10% inulin). No fermentation was done; thus the toast obtained was treated at 220 °C during 20 min.

In vitro digestion model

The procedure was adapted from the method outlined by Gil-Izquierdo *et al.*[19] with slight modifications. The method consists of two sequential steps: an initial saliva/pepsin/HCl digestion for 2 h at 37 °C, to simulate the mouth and the gastric conditions, followed by a digestion with bile salts/pancreatin for 2 h at 37 °C to simulate duodenal digestion. Then samples of wheat crispy bread (3 g) were spiked with ENs **A**, $\mathbf{A_1}$, **B**, and $\mathbf{B_1}$ at 1.5 and 3.0 μM concentrations, using a stock methanolic solution (1000 μM) of each EN. After 12 h of contact at room temperature to completely remove the solvent, the samples were then mixed with 6 mL of artificial saliva composed of: KCl 89.6 g L^{-1}, KSCN 20 g L^{-1}, NaH_2PO_4 88.8 g L^{-1}, Na_2SO_4 57 g L^{-1}, NaCl 175.3 g L^{-1}, $NaHCO_3$ 84.7 g L^{-1}, urea 25 g L^{-1}, and 290 mg of α-amylase. The pH of this solution was corrected at 6.8 with HCl 0.1 N.

The mixture composed by the food and the artificial saliva was placed in a plastic bag containing 40 mL of water and was homogenised by a Stomacher IUL Instrument (Barcelona, Spain) for a duration of 30 s.

To this mixture, 0.5 g of pepsin (14.800 U) dissolved in HCl 0.1 N was added. The pH of the mixture was corrected at a value of 2 with HCl 6 N, and then incubated in an orbital shaker (250 rpm) (Infors AG CH-4103, Bottmingen, Switzerland) for 2 h at 37 °C.

After the gastric digestion, the duodenal digestion was simulated. The pH was increased to 6.5 with $NaHCO_3$ (0.5 N) and then 5 mL (1 : 1; v/v) of pancreatin (8 mg mL^{-1})–bile salts (50 mg ml^{-1}), dissolved in 20 mL of water were added and incubated in a 37 °C shaking orbital (250 rpm) for 2 h.

After this last step of digestion, the mixture was centrifuged at 4000 rpm for a duration of 1 h at 4 °C.

The schematic representation of the *in vitro* digestion model is represented in Fig. 3.

Analysis of ENs

ENs contained in the duodenal fluids were extracted as follows:[19] 5 mL of each mixture previously described was placed in a 20 mL test tube, and extracted three times with

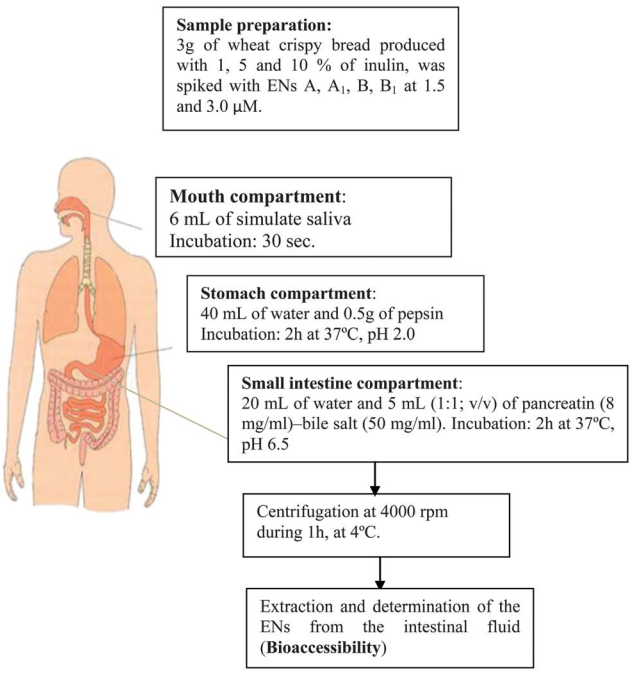

Fig. 3 Schematic representation of the *in vitro* digestion model that simulates the physiology of the digestive processes in the mouth, stomach and small intestine.

5 mL of ethyl acetate utilizing a vortex VWR international (Barcelona, Spain) for 1 min. The organic layers were mixed, centrifuged (Centrifuge 5810R, Eppendorf, Germany) at 4000 rpm and 4 °C for 10 min and were then evaporated by a rotary evaporator (Buchi, Switzerland), operating at 30 °C and at the pressure of 30 mbar. The ENs were then resuspended in 1 mL of methanol.

LC analyses of ENs were performed using LC-10AD (Shimadzu, Japan) pumps and a diode array detector (DAD) Shimadzu (Japan). A Gemini (150 × 4.6 mm, 5 μm) Phenomenex column was used. LC conditions were set up using a constant flow rate of 1.0 mL min^{-1} for the mobile phase. The starting ratio of acetonitrile–water (70 : 30 v/v) was kept constant for 5 min and was then linearly modified to 90% acetonitrile in 10 min. After 1 min the mobile phase was taken back to the starting conditions in 4 min. ENs were detected at 205 nm. All samples were filtered through a 0.22 μm syringe filter Phenomenex prior to injection (20 μL) onto the column. Mycotoxin identification was performed by comparing retention times and UV spectra of purified samples to pure standards. A further confirming action was performed by coinjecting pure standards together with each sample. Quantification of mycotoxins was carried out by comparing peak areas of investigated samples to the calibration curve of the standards.[18]

Calculation and statistical analysis

All experiments were performed three times. Statistical analysis was carried out using analysis of variance (ANOVA), followed by Dunnet's multiple comparison tests. Differences were considered significant if $P < 0.05$.

Results

Method performance

Mean recoveries were operated on the fortified intestinal fluid (free from the contamination of ENs) ($n = 5$) at different levels of ENs **A**, **A_1**, **B**, **B_1** (0.3–50 μg g^{-1}). Table 1 shows that the ENs recoveries ranged from 84.2 ± 4.3% for the EN **A_1** to 89.5 ± 3.1% of EN **B_1**. Important recovery data were also evidenced for the EN **B** and **A** with 86.6 ± 2.7 and 88.6 ± 2.4% respectively. Intra-day ($n = 5$) and inter-day (5 different days) variation values ranged from 1.8 to 3.1% respectively for ENs. These values are below 15% which is the maximum variation for certification exercises for several mycotoxins. The detection limit (LOD) and the limit of quantification (LOQ) values were calculated according to $s/n = 3$ and $s/n = 10$, respectively. The LODs and the LOQs of ENs are 215 and 600 μg kg^{-1} for EN **A**, 140 and 400 μg kg^{-1} for EN **A_1**, 145 and 400 μg kg^{-1} for EN **B** and finally 165 and 500 μg kg^{-1} for EN **B_1** (Table 1).

Bioaccessibility of ENs

The bioaccessible fraction is considered the maximum amount of contaminant available for absorption in the body. The *in vitro* digestion model was useful for determining the bioaccessibility of mycotoxins from food.

LC-DAD chromatograms of the ENs present in the duodenal fluid is shown in Fig. 4. As evidenced in Table 2, the mean value of duodenal bioaccessibility in the wheat crispy bread produced without the alimentary fiber inulin was 76%, considering the two concentration of ENs utilized in this study. The highest bioaccessibility value was evidenced in the wheat crispy bread contaminated with 1.5 μM of EN **A**, with an 85% bioaccessibility, whereas the lowest was evidenced in the assay carried out with EN **B** at 1.5 μM concentration with a 69% bioaccessibility.

As regards the wheat crispy breads produced with 1% of inulin, the bioaccessibility values for all the mycotoxins of this study are not statistically different from those obtained in the experiment performed without inulin. In particular, the mean bioaccessibility value evidenced in this part of the study was of 74%. Also in this part of the experiment the highest bioaccessibility value was evidenced for the sample contaminated with the EN **A** (1.5 μM) with 83% bioaccessibility. The lowest value was evidenced in the same sample spiked with 3.0 μM of EN **A**, where at the end of the simulated gastrointestinal digestion the bioaccessibility data was 65%.

Very different is the phenomenon observed for the sample produced with 5% of inulin, where early on all the bioaccessibility values evidenced for the mycotoxins employed in this study (except the samples contaminated with ENs **A_1** and **B_1** at 1.5 μM) were statistically different from the control (wheat crispy bread produced without inulin). The mean bioaccessibility value for the samples contaminated with ENs and treated with a simulated digestion model was 66%. The wheat crispy breads contaminated with ENs **A** (1.5 μM), **A_1** (3 μM) and **B_1** (1.5 and 3.0 μM) were statistically different compared with the control ($P \leq 0.05$) with a mean bioaccessibility value of 68%. Also the samples spiked with ENs **A** and **B** respectively, at 3.0 μM, were

Table 1 Mean recoveries, inter-day and intra-day variations, LOD and LOQ of the analytical method applied to determine the ENs in the bioacccesible fraction

Mycotoxin	Mean recovery ± RSD (%)	Inter-day variation (%)	Intra-day variation (%)	LOD (μg kg^{-1})	LOQ (μg kg^{-1})
EN **A**	88.6 ± 2.4	3.1	9.7	215	600
EN $\mathbf{A_1}$	84.2 ± 4.3	2.0	6.4	140	400
EN **B**	86.6 ± 2.7	1.8	9.7	145	400
EN $\mathbf{B_1}$	89.5 ± 3.1	1.9	10.1	165	500

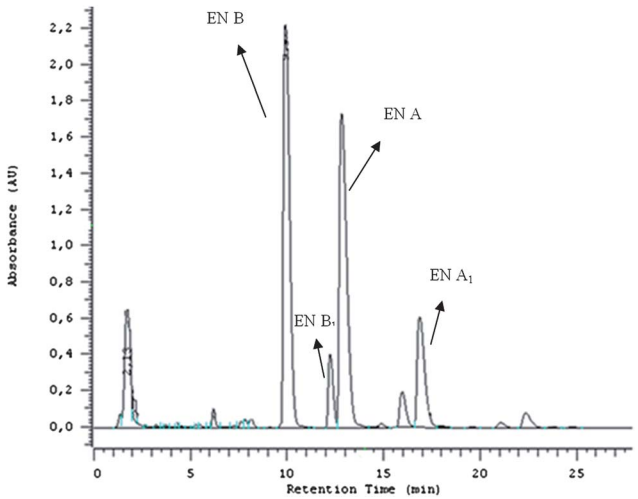

Fig. 4 LC-DAD chromatograms of the ENs: **B**, $\mathbf{B_1}$, **A**, $\mathbf{A_1}$ present in the duodenal fluid, originated by a gastrointestinal simulated digestion of a wheat crispy bread.

statistically different ($P \leq 0.001$) with respect to the wheat crispy bread produced without the alimentary fiber inulin.

In the crispy bread produced with 5% of inulin the highest and lowest bioaccessibility data were evidenced in the assays carried out with ENs $\mathbf{B_1}$ (1.5 μM) and $\mathbf{A_1}$ (3 μM), obtaining 74 and 58% bioaccessibility, respectively.

If we focus our attention on the experiment operated with 10% of inulin, it is possible to observe in Table 2, that all the bioaccessibility data obtained in this part of the study, excluding the values related to the assays carried out with EN $\mathbf{A_1}$ (1.5 μM), were statistically different from the control. In particular, the mean bioaccessibility value evidenced, considering all the ENs used in this study at the two concentrations tested was 63%. The highest reduction of the ENs contained in the duodenal fluid was observed in the experiment realized with EN **A** (3.0 μM), obtaining a 51% bioaccessibility ($P \leq 0.000$). Good reduction of the ENs contained in the bioaccessible fraction was also evidenced in the experiments operated with EN **A** (1.5 μM), $\mathbf{A_1}$ (3 μM) and **B** (3 μM) with bioaccessibilities of 59, 56 and 57%, respectively ($P \leq 0.05$). The highest value of the ENs bioaccessibility (74%) was obtained for the sample contaminated with EN **B** (1.5 μM).

Discussions

Bioaccessibility has been defined as the fraction of a compound that is released from its matrix in the gastrointestinal tract and thus becomes available for intestinal absorption (*i.e.*, enters the blood stream). Bioaccessibility includes the entire sequence of events that takes place during the digestive transformation of food into material that can be assimilated by the body, the absorption/assimilation into the cells of the intestinal epithelium, and lastly, the presystemic metabolism (both intestinal and hepatic). Bioaccessibility analyses can be approached using general experimental techniques (there are systematic techniques common to all types of foods) that can be adapted to all types of claims regarding nutritional content.[20]

All the intestinal fluids originating from the digestion of food can contain some toxic compounds that pass without structural modifications by enzymatic attack of the gastrointestinal digestion and that can interact with the intestinal epithelium starting a process of degradation of the transepithelial membrane along the entire gastrointestinal tract. This study demonstrates that the quantity of ENs that arrive in contact with the transepithelial membrane in the crispy breads produced with 5 and 10% of the dietary fiber inulin is lower than the quantity evidenced in the products produced with 1% and without inulin.

In order to maintain an effective barrier function, epithelia need to exist in a constant state of regeneration. The intestinal epithelium rapidly regenerates its entire tissue entity throughout life, in approximately one week. Mature cells derived from intestinal stem cells migrate upward along the crypt-villus axis towards the tip of the villus, gradually differentiating as they come closer to the tip.[21]

The cytotoxicity effect of ENs **A**, $\mathbf{A_1}$, $\mathbf{A_2}$, **B**, $\mathbf{B_1}$, $\mathbf{B_4}$ and $\mathbf{J_3}$ was compared on three tumour cell lines, the human epithelial colorectal adenocarcinoma (Caco-2), the human colon carcinoma (HT-29), and the human liver carcinoma (Hep-G2). The endpoint evaluated was the mitochondrial integrity by using the MTT assays, after 24 and 48 h of incubation.[26]

The half maximal inhibitory concentration (IC_{50}) value for EN $\mathbf{A_2}$ on Caco-2 cells, after 24 h exposure, was 18.7 ± 4.5 μM and decreased to 2.6 ± 0.7 μM after 48 h of incubation. However, ENs **A**, $\mathbf{A_1}$, $\mathbf{B_1}$ and $\mathbf{B_4}$ exert pronounced cytotoxic effects in all the cell lines tested by the MTT assay after 24 and 48 h of incubation. EN $\mathbf{A_1}$ was found to be the most cytotoxic EN tested.[26]

This study can be considered as the first in which the bioaccessibility of ENs was related to the quantity of fiber present in a food product. It also demonstrates that in food there are some bioactive compounds present as alimentary fiber inulin, which can naturally reduce the bioaccessibility of toxic compounds, such as ENs, and can also the reduce the risk associated with the interaction of these molecules with the intestinal epithelium.

Table 2 Bioaccessibility of ENs from wheat crispy bread produced with a) 0% of inulin, b) 1% of inulin, c) 5% of inulin, d) 10% of inulin. Concentration of 1.5 and 3.0 μM of ENs were added to 3 g of food. Data are (expressed as) ± SD ($n = 3$). Significantly different from the control, $P \leq 0.05$ (*), $P \leq 0.001$ (**), $P \leq 0.000$ (***)

		Gastrointestinal digestion	
Mycotoxin	Concentration added μM	Conc. (μM)	Bioaccessibility (%)
(a) 0% of inulin			
EN **A**	1.5	1.27 ± 0.11	85
	3.0	2.66 ± 0.13	87
EN $\mathbf{A_1}$	1.5	1.09 ± 0.13	73
	3.0	2.10 ± 0.22	70
EN **B**	1.5	1.03 ± 0.14	69
	3.0	2.10 ± 0.22	73
EN $\mathbf{B_1}$	1.5	1.11 ± 0.18	74
	3.0	2.22 ± 0.25	74
(b) 1% of inulin			
EN **A**	1.5	1.24 ± 0.08	83
	3.0	1.96 ± 0.03	65*
EN $\mathbf{A_1}$	1.5	1.07 ± 0.03	72
	3.0	2.19 ± 0.01	73
EN **B**	1.5	1.09 ± 0.02	73
	3.0	2.03 ± 0.09	68
EN $\mathbf{B_1}$	1.5	1.1 4 ± 0.02	76
	3.0	2.27 ± 0.08	76
(c) 5% of inulin			
EN **A**	1.5	1.10 ± 0.03	73*
	3.0	1.90 ± 0.02	63**
EN $\mathbf{A_1}$	1.5	0.96 ± 0.01	64
	3.0	1.74 ± 0.03	58*
EN **B**	1.5	1.05 ± 0.03	70
	3.0	1.77 ± 0.03	59**
EN B_1	1.5	1.11 ± 0.01	74*
	3.0	2.04 ± 0.03	68*
(d) 10% of inulin			
EN **A**	1.5	0.89 ± 0.03	59*
	3.0	1.54 ± 0.03	51***
EN $\mathbf{A_1}$	1.5	0.92 ± 0.01	61
	3.0	1.70 ± 0.03	56*
EN **B**	1.5	1.10 ± 0.03	73*
	3.0	1.72 ± 0.03	57*
EN $\mathbf{B_1}$	1.5	1.11 ± 0.01	74*
	3.0	1.90 ± 0.03	63*

The dietary fiber used in this study, inulin, has a much lower caloric value than typical carbohydrates, which is due to the β (2,1)-bonds linking the fructose molecules. Since these bonds render them indigestible by mammalian digestive enzymes, they pass to the large intestine intact and are then fermented by the colonic bacteria. In a human study conducted by Seçkin *et al.*[28] it was found that 86–88% of the dose (10, 17, 30 g) of inulin was recovered in the ileum effluents, which thereby supported the above statements. The energy derived from fermentation is largely a result of the production of short chain fatty acids and lactate, which are metabolized and contribute 1.5 kcal g^{-1} (6.3 kJ g^{-1}) of useful energy, rather than 4 kcal g^{-1} from its monosaccharide composition.[29] It is due to this property that inulin can pass unmodified into the gastrointestinal tract to be fermented by the colonic bacteria can explicate the fact that the product produced in this study, with high inulin concentrations, has a bioaccessibility of ENs that is only minor. It is possible that the no digested inulin in the gastrointestinal fluid can form a reticular structure which has the properties to retain some bioactive compounds, like ENs, and that this property is proportional to the concentration of the fiber present.

Avantaggiato *et al.*[30] studied the intestinal absorption of zearalenone (ZEA) by using a laboratory model that mimics the metabolic processes of the gastrointestinal tract of healthy pigs. Approximately 32% of ZEA intake (247 mg) was released from the food matrix to the bioaccessible fraction during 6 h of digestion and was rapidly absorbed at intestinal level.

Carolien *et al.*[20] described the applicability of an *in vitro* digestion model allowing for measurement of the bioaccessibility of ingested mycotoxins from food as an indicator of oral bioavailability of the AFB1 from peanut slurry and of the OTA from buckwheat, evidencing bioaccessibility data of 94% and 100%, respectively. Considering our bioaccessibility values, these mycotoxins can be considered as 20 to 25% more bioaccessible with respect to the ENs studied in the present work.

Avantaggiato *et al.*,[31] utilizing a laboratory model, set to simulate the *in vivo* conditions of the porcine gastrointestinal tract, the small intestinal absorption of the fumonisins B_1 and B_2 (FB_1 and FB_2), OTA, deoxinivalenol (DON), AFB_1 and ZEA contained in different feeds in the presence or absence of sequestering materials.

When no sequestering material was added to the feed (control), the total intestinal absorptions of mycotoxins (corresponding to the mycotoxin amounts measured in jejunal plus ileal dialysate fluids) was 105% for FB_1, 89% for FB_2, 87% for OTA, 74% for DON, 44% for AFB1, and 25% for ZEA.

Recently[32] the bioaccessibility of AFB1 and OTA was determined in pistachio nuts, buckwheat, and in infant food using an *in vitro* model under fed conditions. The average bioaccessibility of AFB1 and OTA was about 90%, and 30%, respectively, depending on several factors, such as food product, contamination level, compound and type of contamination (spiked *versus* naturally contaminated). The bioaccessibility of AFB1 was 15% higher than the mean value obtained for the ENs in our study, whereas the OTA bioaccessibility was 30% lower.

Acknowledgements

This research was supported by the Ministry of Science and Innovation (AGL2010-17024), and by the pre PhD program of University of Valencia "Cinc Segles".

References

1 L. Ivanova, E. Eystein Skjerve, G. S. Eriksen and S. Uhlig, *Toxicon*, 2006, **47**, 868–876.
2 N. E. Zhukhlistova, G. N. Tishchenko, I. V. Tolstykh and V. A. Zenkova, *Crystallogr. Rep.*, 1999, **44**, 8–12.
3 L. Supothina, S. M. Isaka, K. Kirtikara, M. Tanticharoen and Y. Thebtaranonth, *J. Antibiot.*, 2004, **57**, 732–738.
4 R. D. Plattner and P. E. Nelson, *Microbiology*, 2004, **60**, 3894–3896.
5 H. Tomoda, X. H. Huang, J. Cao, H. Nishida, R. Nagao, S. Okuda, H. Tanaka, S. Omura, H. Arai and K. Inoue, *J. Antibiot.*, 1992, **45**, 1626–1632.
6 M. Kamyar, P. Rawnduzi, C. R. Studenik, K. Kouri and R. Lemmens-Gruber, *Arch. Biochem. Biophys.*, 2004, **429**, 215–223.
7 S. Uhlig, M. Torp and T. B. Heier, *Food Chem.*, 2006, **94**, 193–201.
8 K. Hiraga, S. Yamamoto, H. Fukuda, N. Hamanaka and K. Oda, *Biochem. Biophys. Res. Commun.*, 2005, **328**, 1119–1125.
9 H. S. Lee, H. H. Song, J. H. Jeong, C. G. Shin, S. U. Choi and C. Lee, *Toxicon*, 2008, **51**, 1178–1185.
10 E. Fernández-García, I. Carvajal-Lérida and A. Pérez-Gálvez, *Nutr. Res.*, 2009, **29**, 751–760.
11 E. H. Hipsley, *Br. Med. J.*, 1953, **2**, 420–422.
12 A. S. Truswell, *Eur. J. Clin. Nutr.*, 1995, **44**, 105–109.
13 K. K. Nair, S. Kharb and D. K. Thompkinsont, *Food Rev. Int.*, 2010, **26**, 189–203.
14 N. M. Delzenne, C. Daubioul, A. Neyrinck, M. Lasa and H. S. Taper, *Br. J. Nutr.*, 2000, **87**, 255–259.
15 R. J. Redgwell and M. Fischer, *Mol. Nutr. Food Res.*, 2005, **49**, 521–535.
16 J. Van Loo, P. Coussement, L. De Leenheer, H. Hoebregs and G. Smits, *Crit. Rev. Food Sci. Nutr.*, 1995, **35**, 525–552.
17 Z. Madar and H. S. Odes, Dietary fiber in metabolic diseases, in *Dietary fiber research*, ed. R. Paoletti, Karger, Basel, CH, 1990, pp. 1–65.
18 G. Meca, M. J. Ruiz, J. M. Soriano, A. Ritieni, A. Moretti, G. Font and J. Mañes, *Toxicon*, 2010, **56**, 418–424.
19 A. Gil-Izquierdo, P. Zafrilla and F. A. Tomás-Barberá, *Eur. Food Res. Technol.*, 2002, **214**, 155–159.
20 H. M. Carolien, H. Versantvoort, A. G. Oomen, E. Van de Kamp, C. J. M. Rompelberg and J. A. M. Adrienne, *Food Chem. Toxicol.*, 2005, **43**, 31–40.
21 C. Booth and C. S. Potten, *J. Clin. Invest.*, 2000, **105**, 1493–1499.
22 G. Brunsgaard, *J. Anim. Sci.*, 1998, **76**, 2787–2798.
23 F. Caloni, G. Ranzenigo, F. Cremonesi and L. J. Spicer, *Toxicon*, 2009, **54**, 337–344.
24 A. Kralj, M. Gurgui, G. M. König and G. van Echten-Deckert, *Toxicol. Appl. Pharmacol.*, 2007, **225**, 113–122.
25 V. Berger, A. F. Gabriel, T. Sergent, A. Trouet, Y. Larondelle and Y. J. Schneider, *Toxicol. Lett.*, 2003, **140**, 465–476.
26 G. Meca, G. Font and M. J. Ruiz, *Food Chem. Toxicol.*, 2011, **49**, 2464–2469.
27 S. Bouhet, E. Le Dorze, S. Peres, J. M. Fairbrother and I. P. Oswald, *Food Chem. Toxicol.*, 2006, **44**, 1768–1773.
28 A. K. Seckin, B. Ergönül, H. Tosun and P. G. Ergönül, *Food Sci. Technol. Res.*, 2010, **6**, 605–612.
29 M. B. Roberfroid, *J. Nutr.*, 1999, **129**, 1398–1401.
30 G. Avantaggiato, R. Havenaar and A. Visconti, *Food Chem. Toxicol.*, 2003, **41**, 1283–1290.
31 G. Avantaggiato, R. Havenaar and A. Visconti, *J. Agric. Food Chem.*, 2007, **55**, 4810–4819.
32 B. Kabak, E. F. A. Brandon, I. Var, M. Blokland and A. J. A. M. Sips, *J. Environ. Sci. Health, Part B*, 2009, **44**, 472–480.

